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THE VISCERO-PANNICULAR REFLEX

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Received for publication April 13, 1935

In the course of experiments on visceral pain, we observed a reaction which seems quite valuable for the study of the conduction of centripetal impulses from the viscera. This reaction can be very easily produced and observed. It is very constant and it is present even when other reactions accompanying visceral stimulation can no longer be elicited.

The first observation was made in a decerebrate cat in which a balloon was tied into the gall bladder. This balloon was connected by a T-tube to a manometer and a rubber bulb for producing pressure. On dilatation of the gall bladder by the balloon, a movement of the skin in the lateral and dorsal parts of the thorax was observed. It was at first supposed that this might be a manifestation of the viscero-pilomotor reflex. However, further analysis on 34 cats showed that the reaction still persists after the hair has been removed by the cutting or shaving, and also persists after the intercostal nerves which supply the skin with vegetative fibers are severed. In a shaved animal one may readily observe that the skin moves on the underlying tissues when the gall bladder is stimulated. This movement is mainly in a longitudinal direction craniad, with a transverse dorsad component which is most marked in the region immediately below the shoulder. It is bilateral and often more distinct on the right side than on the left.

If the skin is separated carefully from the underlying tissues, after a median incision on the sternum, it is easily seen that this reaction is due to a contraction of the thin sheet of muscle lying immediately under the skin, which is known as the panniculus carnosus. This contraction of the panniculus carnosus may be observed as well when the fibers are adhering only to the skin as when they are still in contact with the underlying trunk muscles. Since we are dealing with a visceral reflex which has for its

effector the panniculus carnosus, we have named it the viscero-pannicular reflex.

If the animal is in good condition, the contraction of the pannicular muscle may be maintained as long as the visceral stimulation is continued, and it has the character of a tonic or mixed tonic-clonic reaction (figs. 1 and 2). This is shown by records obtained by sewing a string into the

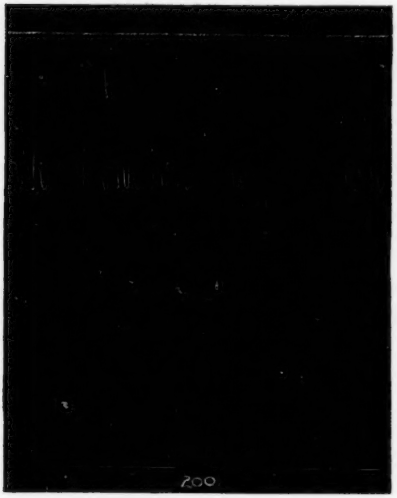

Fig. 1

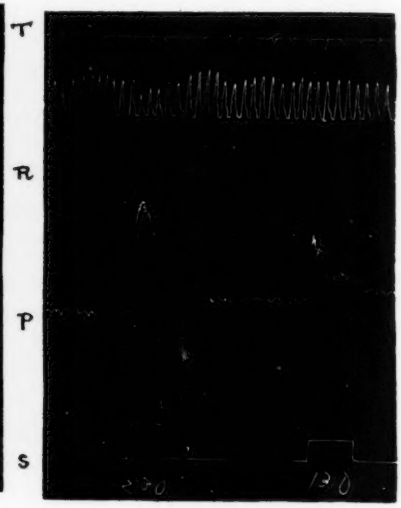

Fig. 2

Fig. 1. Decerebrate cat. *P*, contraction of the panniculus carnosus; *R*, respiration (tambour connected with tracheal cannula); *S*, stimulus (pressure on balloon in gall bladder in millimeters mercury); *T*, time in seconds.

Fig. 2. Decerebrate cat. *P*, contraction of the panniculus carnosus; *R*, respiration; *S*, stimulation; *T*, time in 5 second intervals.

First stimulation (pressure 230 mm. Hg) elicits contraction of the panniculus carnosus and inhibition of respiration; 2nd stimulation (130 mm. Hg) produces weaker contraction of the panniculus carnosus, but no change in respiration.

skin of the dorsal part of the thorax, and connecting this with a muscle lever. In some records, the skin was intact, while in others it was separated from the underlying tissues. Simultaneous records of respiration showed that the viscero-pannicular reflex is often present when visceral stimulation fails to elicit a change in respiration (fig. 2). The maximum pressure employed in stimulation of the gall bladder was 350 mm. mercury, while the minimum effective pressure was usually 80 mm. mercury, al-

though a lower pressure (15 mm. mercury) was occasionally sufficient to evoke a response.[1]

A similar but weaker response was obtained by distention of the duodenum, while dilatation of the urinary bladder failed to give a definite reaction.

When the right splanchnic nerve is severed in the abdomen or in the thorax, stimulation of the gall bladder no longer evokes a response. This is in agreement with the statements of Schrager and Ivy (1928) and of Davis, Pollock and Stone (1932) who found, in a study of the respiratory reaction to dilatation of the gall bladder and the biliary ducts, that these reactions are abolished by cutting the right splanchnic nerve.

Faradic stimulation of the central end of the right splanchnic nerve elicits a distinct contraction of the panniculus carnosus even before decerebration, but after decerebration the reaction is increased and can be elicited with weaker stimuli than before decerebration. On faradic stimulation of the left splanchnic nerve, with the brain intact and under superficial ether anesthesia, a slight movement of the skin of the homolateral side can be obtained. This reaction, however, is much more marked after decerebration.

These observations indicate that the centripetal part of the viscero-pannicular reflex arc is by way of the splanchnic nerve.

The central part of the reflex arc is located entirely within the spinal cord. The higher parts of the central nervous system are not necessary for the maintenance of this reflex, since it is still present after transverse section between the medulla oblongata and the spinal cord. The centrifugal part of the reflex arc is by way of the eighth cervical and first thoracic nerves, and then by the anterior thoracic nerves, since severing these nerves abolishes the reflex. This is in agreement with the statement of Langworthy (1924) that the panniculus carnosus in the cat is supplied by the nervi thoracales anteriores.

SUMMARY

In decerebrate cats, distention of the gall bladder or duodenum or faradic stimulation of the splanchnic nerves evokes a contraction of the panniculus carnosus muscle (viscero-pannicular reflex) which results in a movement of

[1] These variations in the minimum amount of pressure required to evoke a response on stimulation of the gall bladder may be related to the great variability which was observed in the size and other characteristics of the gall bladder. It must also be noted that the elasticity of the balloon plays a part in determining the minimum effective stimulating pressure, since a part of the stimulating pressure is always employed in overcoming this elasticity.

the skin of the trunk on the underlying tissues. This reaction is a spinal cord reflex, with its afferent pathway through the splanchnic nerve and its centrifugal pathway through the nervi thoracales anteriores.

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DAVIS, L., L. J. POLLOCK AND T. T. STONE. Ibid. **55**: 418, 1932.
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THE SUBSTANCE IN HUMAN SEMINAL FLUID AFFECTING UTERINE MUSCLE[1]

JESSIE REED COCKRILL, EDGAR G. MILLER, JR. AND RAPHAEL KURZROK

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Received for publication April 25, 1935

It was reported by Kurzrok and Lieb (1) that human semen affected human uterine muscle. Normally, the effect was relaxation of the muscle. In certain cases (depending apparently on differences in the semen or in the uterus or in both) the effect was contraction. These effects have been demonstrated both *in vitro* and *in vivo*. The clinical data in the cases studied have shown the probability of a relation of this phenomenon to certain aspects of the problem of sterility.

The present studies were undertaken to identify the substance, in semen, responsible for this pharmacologic action.

Muscle strips from more than 400 human uteri obtained at operations in Sloane Hospital, and more than 200 specimens of seminal fluid, obtained from 75 patients in the Vanderbilt Clinic and private patients, were used. The semen samples from 65 of these patients caused relaxation of uterine muscle (fig. 1). Each sample was tested on strips from different uteri. In these relaxing cases it was found that, while the semen relaxed most uteri, at least one uterus would respond by contraction. Samples from 10 patients of the series were, on the contrary, found always to cause contraction (figs. 2 and 3).

The tests were done by the usual kymograph technique. The muscle strips were approximately 1 cm. in length and 1 gram in weight, and were suspended in 100 cc. of oxygenated Ringer solution at 37.5°C. The amount of seminal fluid added to this was usually 0.4 cc. or its equivalent.

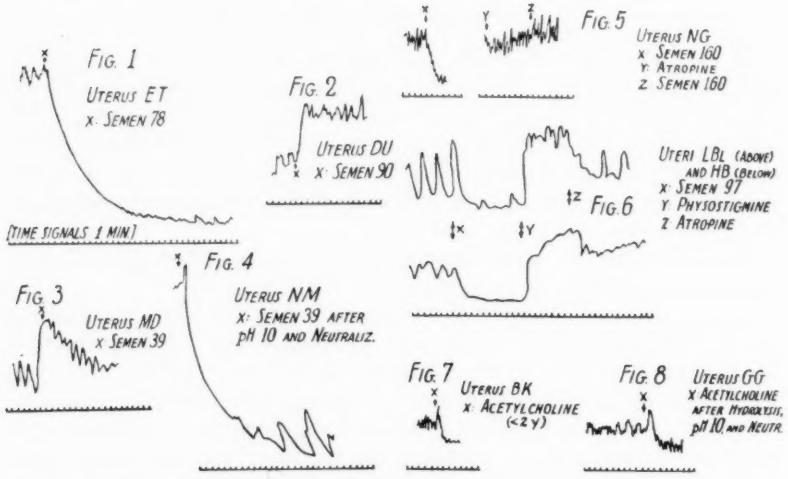
The contracting substance was first studied. Its activity slowly diminished with time at room temperature. Contracting semen, brought to pH 9.5 to 10 by addition of 0.4 N sodium hydroxide, allowed to stand for 30 minutes, and then neutralized, caused relaxation of uterine muscle (figs. 3 and 4). Similarly treated at pH 11 or above, it became inactive. Boiling the semen destroyed its activity. The active substance was found

[1] This work was aided by a grant from E. R. Squibb & Sons.

to be dialyzable through parchment or collodion membranes. It was soluble in water or in 95 per cent alcohol. An active fraction, prepared by drying, alcohol extraction, evaporation, and washing with dry ether, gave a negative biuret reaction.

Physostigmine, which itself had no pharmacologic effect on the muscle, enhanced the contracting action of the semen. Atropine abolished the action without altering the normal rhythm of the muscle.

Contracting semen which had been inactivated by boiling was treated with acetyl chloride. This gave a restored and enhanced contracting action. Esterase, prepared from pig liver, was shown to greatly diminish the activity. We suggest that our inability to abolish it completely



with esterase may have been due to the fact that the high level of pH necessary for complete hydrolysis could not be maintained on account of its effect *per se* on the active substance.

These results indicate that the contracting substance is acetyl choline or some very similar material. The pharmacologic data can be duplicated in detail with human uterine muscle, using pure acetyl choline. The amount present in semen is too small for chemical isolation or determination, but comparative dosage data suggest that the concentration in a uterus-contracting semen is of the order of about 0.01 mgm. in 1 cc.

Our experiments with the seminal samples causing relaxation of uterine muscle have led us to the tentative conclusion that the relaxing action is also due to acetyl choline in smaller dosage.

It appears that all semen specimens contain some of the contracting substance (acetyl choline), since normal relaxing samples could be shown

to cause contraction of some uteri. Also, when a specimen which relaxed most uteri was dried and the alcohol-soluble fraction from it concentrated and used in larger doses, it then caused contraction. Uteri of other animals (guinea pig, rabbit) which are extremely sensitive to acetyl choline, were invariably contracted by human semen. Relaxing semen gave an acetyl choline action on rabbit intestine.

The relaxing substance had the same solubilities as the contracting. It was heat labile, slowly decreased with time in water solution, and was destroyed by treatment with sodium hydroxide (pH 11) for 30 minutes. Esterase definitely lessened the activity.

Atropine (in amounts which alone had no effect on the muscle) abolished the relaxing effect of the semen (fig. 5). Addition of physostigmine to a relaxing semen invariably reversed the action, producing a contraction (fig. 6). This contraction was abolished by atropine.

Relaxing semen (1 cc.) caused a fall in the blood pressure of a pithed cat comparable to 0.001 mgm. of acetyl choline.[2]

Efforts to produce relaxation of uterine muscle with pure acetyl choline in exceedingly small doses (a few gammas) were several times successful (fig. 7). Dilute aqueous solutions of acetyl choline, treated with sodium hydroxide (pH 10) for 10 minutes, and then neutralized, caused definite relaxation (fig. 8).

Another property of semen and of acetyl choline was noted. Many uterine strips which were completely inert when suspended in the Ringer solution, giving a straight line record on the kymograph paper, promptly assumed a normal activity with normal rhythm after treatment with these materials; also, there was frequently a definite improvement in the rhythm and tone of active strips.

These facts, together with the apparent relation of uterine relaxation or contraction to the acceptance or expulsion of the sperm, suggest an important physiologic rôle for acetyl choline in semen.

In our series there were a few interesting variants from the general results. Occasionally a semen was found to abolish the normal rhythm of the muscle, but not to alter the tone. This type, retested on the same muscle after alkali treatment (pH 10) and neutralization, gave marked relaxation. These results were considered to be a function of the amount of acetyl choline present. Several semen samples produced no effect when tested on many different uteri. These specimens were definitely abnormal in showing only a few, non-motile, sperm. Other specimens showing the same morphological abnormality gave normal pharmacologic effects.

The factors conditioning the responses of uterine muscle to the action of acetyl choline and other substances are being investigated.

[2] This test was made by Doctor Mulinos, of the Department of Pharmacology.

We are indebted to Professor Watson for making available the clinical facilities of Sloane Hospital and Vanderbilt Clinic, and to Professors Lieb and Mulinos, of the Department of Pharmacology, for help and advice.

SUMMARY

Acetyl choline or some similar substance is present in human seminal fluid. When present in unduly large amounts it causes contraction of human uterine muscle. It is probably also responsible for the normal relaxation of uterine muscle. Its pharmacologic activity is of importance in the physiology of sperm entry into the uterus.

REFERENCE

(1) KURZROK, R. AND C. C. LIEB. Proc. Soc. Exp. Biol. Med. **28**: 268, 1930.

A COMPARATIVE STUDY OF SODIUM, CHLORIDE AND BLOOD PRESSURE CHANGES INDUCED BY ADRENAL INSUFFICIENCY, TRAUMA AND INTRAPERITONEAL ADMINISTRATION OF GLUCOSE[1,2]

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Received for publication May 1, 1935

Several investigators have recently emphasized the importance of certain specific fluid and electrolyte changes associated with adrenal insufficiency (Swingle et al., 1933–34a, b, c, d; Loeb et al., 1933a, b; Harrop et al., 1933a, b, c; Zwemer and Sullivan, 1934). Sodium, fluid and circulatory changes analogous to those characteristic of adrenal insufficiency have also been shown to follow intraperitoneal injections of large quantities of glucose (Gilman, 1934).

Many of the signs and symptoms of secondary shock are strikingly similar to those induced by adrenal insufficiency and also by intraperitoneal glucose injections. It seemed important, therefore, to determine whether serum sodium, chloride and arterial pressure changes in traumatic shock might not also be comparable, and to what extent certain phenomena following trauma and glucose administration were influenced by the presence or absence of adequate cortical hormone.

The type of adrenalectomized dog used for experimental purposes in this laboratory has been previously described (Swingle et al., 1933–34). All dogs employed were in normal health and nutritive condition, and the adrenalectomized animals had been operated for periods varying from two months to one year previous to use in these experiments. The maintenance dose of adrenal cortical hormone for the adrenalectomized animals was 0.1 to 0.2 cc. per kgm. per day unless otherwise stated. The dosage used for reviving the animals from shock and collapse was 3 cc. per kgm. given daily in divided doses until the blood pressure and electrolyte pattern had returned to normal. Mean blood pressure was determined by direct needle puncture into the femoral artery (Parkins, 1934). Serum sodium was determined by the method of Butler and Tuthill (1931) and

[1] These investigations have been aided by a grant from The Rockefeller Foundation.

[2] We are indebted to Parke Davis & Co. for considerable quantities of fresh beef adrenals used in preparation of cortical hormone.

chlorides by that of Van Slyke and Sendroy (1923) as modified by Eisenman (1929). All blood samples were taken from the femoral artery, and were drawn, delivered and centrifuged under oil.

Deep ether anesthesia was used on both normal and adrenalectomized animals subjected to trauma and care taken that the dogs did not suffer pain during the experimental procedure. Muscle trauma to the posterior extremities was produced by the method described in a previous study (Swingle and Parkins, 1935).

I. *A comparison of the serum sodium and chloride changes of adrenal insufficiency with those following trauma to the extremities of the healthy, vigorous adrenalectomized and the unoperated animal.* The serum sodium, chloride and arterial pressure have been followed in 6 adrenalectomized and 5 normal dogs subjected to muscle trauma and have been compared with the electrolyte and arterial pressure changes occurring in a typical case of uncomplicated adrenal insufficiency and recovery on extract treatment. Table 1 gives the pertinent data in representative cases of 1, adrenal insufficiency and recovery; 2, trauma to an adrenalectomized dog and the effects of treatment of the resulting shock with cortical hormone, and 3, the typical electrolyte changes observed in the intact dog following severe trauma.

The magnitude of the serum sodium and chloride decline in adrenal insufficiency observed by us (table 1 A) compares favorably with that reported by Loeb et al. (1933b) and Harrop et al. (1933c) for this species. It is of interest to note that the average decrease of 14.8 M.-eq. of sodium and 11 M.-eq. of chloride which occurs within a period of 6 to 12 hours following trauma to the adrenalectomized dog, maintained in normal health and vigor by adequate injections of cortical hormone, is of the same magnitude (table 1 B) as in adrenal insufficiency following withdrawal of hormone (table 1 A). In the one case (trauma) the sodium fell within a few hours, in the other case (insufficiency) a similar decline required a period of 11 days (dog 35, table 1).

The blood samples were taken from the adrenal insufficient and shocked animals when the arterial pressure had declined to approximately 50 mm. of Hg. At this blood pressure level the general symptoms of secondary (traumatic) shock were quite obvious. Cortical hormone (3 cc. per kgm.) was injected intravenously. One shocked dog from which hormone was purposely withheld died one hour after the blood sample was taken. In all other cases, following hormone injection, the blood pressure slowly began to rise, and the shock symptoms disappeared. A normal level of serum sodium and chloride, blood pressure and pulse rate was reëstablished in a manner similar to that occurring in recovery from uncomplicated adrenal insufficiency (table 1; compare animals 35 and 3).

The normal dog with adrenal glands intact, even though traumatized to a

far greater degree than the equally active and vigorous adrenalectomized animal, nevertheless reveals a comparatively negligible decline in serum sodium (average 5.5 M.-eq.) and chloride (average 3.8 M.-eq.), when in profound shock and collapse. (Table 1. Compare dogs 3 and 10.) It should perhaps be emphasized that previous to traumatization, these unoperated dogs were in no better physiological condition, at any rate so far

TABLE 1

Sodium, chloride, and blood-pressure changes in adrenal insufficiency and trauma

DATE	TIME	BLOOD* PRESSURE	PULSE	SERUM SODIUM	SERUM CHLORIDE	REMARKS
A. Adrenal insufficiency. Dog 35 ♂						
		m.m. Hg	*per min.*	*m.-eq.*	*m.-eq.*	
Jan. 23	a.m.	104	76	145.0	107.2	Extract discontinued
Jan. 29	a.m.	75	120	129.6	97.8	Normal food intake, active
Feb. 4	a.m.	50	60	123.4	97.8	Extract injected. Weak, somewhat spastic
Feb. 8	a.m.	98	68	136.9	107.6	Normal food intake, active and vigorous
Feb. 10	a.m.	102	72	144.6	108.1	Maintenance extract
B. Trauma—Adrenalectomized. Dog 3 ♂						
Dec. 17	10 a.m.	103	96	141.7	107.8	Healthy, active, vigorous
Dec. 17	5 p.m.	62	184			Slight weakness, inactive
Dec. 17	10 p.m.	48	180	125.2	96.8	Shock, weak, spastic, extract injected
Dec. 20	10 a.m.	108	104	135.8	105.6	Appears practically normal, eats full ration
Dec. 23	11 a.m.	106	92	140.1	105.8	Normal, maintenance extract
C. Trauma—Unoperated. Dog 10 ♂						
Nov. 16	11 a.m.	110	92	145.9	108.6	Normal
Nov. 16	6 p.m.	66	212			Mild shock, weak, inactive
Nov. 16	11 p.m.	30	160	141.2	103.0	Deep shock, prostrated

* All dogs were carefully trained for blood-pressure determinations unless otherwise stated.

as activity, vigor, appetite, weight and general health are concerned, than the adrenalectomized dogs on maintenance doses of hormone. Nevertheless, the former obviously possessed some factor which enabled them to maintain a nearly normal serum sodium and chloride concentration in the face of a very severe and extensive trauma, which even in mild degree, produced a critical diminution of these electrolytes in the healthy adrenalectomized dog (table 1 B). This factor, present in the intact, but lacking

in the adrenalectomized animal, we consider to be an adequate reserve of cortical hormone in the glands of the unoperated dog. That such is the case is strongly indicated by the fact, amply demonstrated previously (Swingle and Parkins, 1935) and repeated here in connection with electrolyte studies, that the healthy, vigorous, adrenalectomized dog thrown into profound shock and collapse by mild trauma to one extremity, can easily be restored to normal health by adequate injections of cortical hormone (table 1 B).

Histological examination of the adrenals from dogs which had succumbed to muscle trauma, revealed marked diminution of the cortical lipoid, and hemorrhagic changes indicative of functional stress and strain on the glands (Donahue and Parkins, in press).

Despite the marked differences in serum sodium and chloride concentration revealed by the two types of shocked dogs, the diminution in arterial pressure and the gross symptoms of secondary shock were very much the same in both the traumatized normal and adrenalectomized animals.

In the shocked adrenalectomized animals, the low arterial pressure, hemoconcentration and death are associated primarily with a drastic reduction of the serum sodium and chloride, a generalized shift of fluid and certain electrolytes from the blood stream to the tissues and interstitial tissue spaces, and failure of the blood diluting mechanism. The single traumatized limb was invariably only moderately swollen, as contrasted to the greatly swollen and distended tissue in both posterior extremities of the shocked normal dog. The local loss of fluid into the area of injury following the mild degree of trauma necessary to induce profound shock and collapse in the adrenalectomized dog is apparently a minor factor in blood volume reduction. In the normal dog with adrenals intact this local loss of fluid and whole blood into the severely injured limbs is sufficient to reduce the blood volume to the death level.

Since the traumatized adrenalectomized animals are almost invariably anuric, or practically so, throughout the experimental period, it is obvious that wastage of fluid and salts by way of the kidney is not an important factor in the blood volume and electrolyte reduction.

In the shocked animal with intact adrenals the electrolyte changes are relatively slight as compared to those occurring under similar condition in the adrenalectomized dog. The diluting mechanism remains essentially normal for long periods, as indicated by the prompt blood dilution which follows fluid ingestion by mouth in such animals, contrasted with the shocked adrenalectomized animal where such fluid intake is of no avail.

The investigations of Blalock (1930), Parsons and Phemister (1930), Freedlander and Lenhart (1932) and others, clearly demonstrate that the secondary shock following trauma to muscle masses in normal dogs is associated with a local loss of plasma and whole blood into the injured area,

equivalent to approximately one-half of the total blood volume. These investigators emphasize that this loss is of itself sufficient to cause the low blood pressure and subsequent death of the animal. Observations made in this laboratory on a large series of shocked normal dogs agree with this viewpoint.

II. *Serum sodium, chloride and blood pressure changes resulting from shock following a single stage bilateral adrenalectomy.* The shock syndrome which follows bilateral extirpation of the adrenals at a single stage operation has been adequately described in an earlier communication (Swingle and Parkins, 1935). We are concerned here only with the serum sodium and chloride changes associated with this condition.

TABLE 2

Sodium, chloride, and blood-pressure changes following single stage bilateral adrenalectomy

DOG	DATE	TIME	BLOOD PRESSURE	PULSE	SERUM SODIUM	SERUM CHLORIDE	REMARKS
			m.m.Hg	*per min.*	*m.-eq.*	*m.-eq.*	
14	Jan. 8	9:30 a.m.	116	104	143.9	113.2	Normal, untrained
	Jan. 8	11:40 a.m.	132*	172			10 minutes after 40-minute period for complete op.
	Jan. 8	8:05 p.m.	67	140			Weak, lethargic
	Jan. 9	12:30 a.m.	34	124	133.8	100.2	Prostrate, died 10 minutes later
16	Feb. 3	10:00 p.m.	104	80	145.9	111.0	Normal
	Feb. 4	8:00 a.m.	72	204			Lethargic
	Feb. 4	9:40 p.m.	53	200			Spastic
	Feb. 4	11:35 p.m.	38	160	129.8	102.5	Severe shock, died within 2 hours

Dog 14 was untrained for blood-pressure determination.

* Under nembutal anesthesia.

Table 2, dogs 14 and 16, illustrate representative data obtained from five such experiments. The two cases listed show values nearest the mean. The per cent decrease in both sodium and chloride depended largely upon the severity of the symptoms and blood-pressure level at the time the sample was taken. It will be noted that these electrolytes declined considerably. The blood changes and gross symptoms observed in this type of experimental animal were somewhat similar to those following trauma to an extremity of the adrenalectomized dog. (Compare table 2 and table 1 A and 1 B.)

III. *The effect of intraperitoneal glucose injections upon the serum sodium, chloride and arterial pressure of adrenalectomized and unoperated dogs.* Gilman (1934) demonstrated that when isotonic glucose is injected intraperi-

toneally and later followed by paracentesis, a marked fall in serum sodium and chloride occurs within a few hours. The arterial pressure declines owing to the hemoconcentration induced by the internal fluid and electrolyte shift. Animals so injected were also shown to be extremely sensitive to slight hemorrhage and to exhibit other changes analogous to adrenal insufficiency.

In view of the theory of adrenal cortical function adopted by workers in this laboratory (Swingle et al., 1933a, 1934b, c, d), it seemed probable that the resistance of an animal to the electrolyte and fluid shift induced by intraperitoneal glucose administration would depend on the quantity of cortical hormone available, either from the intact adrenals or as supplied by extract injections. It was with this idea in mind that the following experiments were performed on the unanesthetized, intact and adrenalectomized dog.

Rapid intraperitoneal injections of 50 cc. and 100 cc. of 5.5 per cent glucose per kgm. were made through a 12 gauge needle attached by gum tubing to a pressure flask. A local anesthetic was used at the site of a small incision for paracentesis. The rapid removal of an equal volume of fluid by paracentesis was facilitated by use of an aeration tube appropriately connected through a receiving flask to a suction pump.

The pertinent data representing experiments on 8 normal and 8 adrenalectomized dogs are illustrated in table 3. A representative case of the adrenalectomized series (dog 25) collapsed within one hour after the injection of 100 cc. of glucose per kgm. of body weight. The blood pressure was at shock level and the decrease in serum sodium and chloride was comparable to that which occurs over a period of three hours in the representative case of the unoperated series (dog 24) and also in true adrenal insufficiency (table 1 A). It was necessary to withdraw the fluid by paracentesis and inject cortical hormone (3 cc. per kgm.) intravenously to save the adrenalectomized animals. The normal dog, on the other hand, was not so seriously affected, as shown by the 30 mm. decrease in pressure, and the general symptoms (table 3 A) when contrasted to a 60 mm. change and collapse by the adrenalectomized dog (table 3 B). The animals with intact adrenals, although somewhat weakened and lethargic as a result of the hemoconcentration induced by the electrolyte and fluid shift resulting from the glucose injections, were able to withstand a moderate amount of muscle trauma after paracentesis. All recovered from the glucose alone and many from both treatments without hormone injection.

With the injection of 50 cc. of glucose per kgm. the adrenalectomized dog (table 3 D) showed at the end of the 3 hour period sodium and chloride changes comparable to those in the unoperated dog 24 (100 cc. per kgm. and approximately double those produced in the unoperated dog 34 (50 cc. per kgm.). It was unnecessary to withdraw this quantity of fluid from

TABLE 3

*Sodium, chloride, and blood-pressure changes in normal and adrenalectomized dogs following intraperitoneal injection of isotonic glucose**

DATE	TIME	BLOOD PRESSURE	PULSE	SERUM SODIUM	SERUM CHLORIDE	SYMPTOMS	REMARKS
			A. Unoperated. Dog 24 ♂—9.9 kgm. (100 cc. per kilo)				
	hours	*mm. Hg*	*per min.*	*m.-eq.*	*m.-eq.*		
Jan. 2	11:00 a.m.	112	96	141.0	110.0	Normal	Glucose injection
Jan. 2	2:00 p.m.	82	180	124.4	92.0	Depressed, restless	Fluid withdrawn
Jan. 7	10:00 a.m.	110	88	142.6	108.4	Normal	Recovery, paracentesis trauma
			B. Adrenalectomized. Dog 25 ♂—11.7 kgm. (100 cc. per kilo)				
Nov. 30	10:45 a.m.	102	80	139.4		Healthy, active	Min. maint. extract
Nov. 30	11:50 a.m.	42	176	125.6		Collapsed	Fluid withdrawn—Extract
Dec. 6	10:30 a.m.	104	72	139.8		Normal	Healthy, active, vigorous appetite
			C. Unoperated. Dog 34 ♀—9.4 kgm. (50 cc. per kilo)				
Feb. 4	11:45 a.m.	124	120	147.2	113.8	Normal	Untrained for B.P.
Feb. 4	3:00 p.m.	112	108	139.0	106.8	Normal	Vigor, appetite, unaffected
Feb. 6	2:00 p.m.	116	104	146.8	115.6	None externally	Unchanged
			D. Adrenalectomized. Dog 30 ♂—13.0 k. m. (50 cc. per kilo)				
Feb. 2	11:10 a.m.	104	80	142.2	107.8	Normal	Maint. dose hormone
Feb. 2	2:15 p.m.	50	124	125.1	98.7	Weak, spastic	Extract intravenously
Feb. 7	10:00 a.m.	106	72	140.7	106.4	Active vigorous	Excellent condition
			E. Adrenalectomized. Dog 32 ♂—12.5 kgm. (50 cc. per kilo)				
Nov. 19	10:30 a.m.	82	124	144.3		Eats, active	Submin. maint. hormone
Nov. 19	11:30 a.m.	34	102			Complete collapse	No extract injected
Nov. 19	12:40 a.m.	28	92	118.3		Prostrated	Death within 10 minutes

* This table illustrates representative cases from sixteen experiments.

the adrenalectomized animals when adequate hormone was injected intravenously.

The blood sugar elevation (approximately 50 to 100 per cent) induced in the adrenalectomized dog by intraperitoneal glucose injection is quite inadequate to produce any of the injurious effects observed. The deleterious effects resulting from these rapid shifts in fluid and electrolytes and consequent lowering of blood volume and arterial pressure in the healthy adrenalectomized animals (table 3, B and D) are just as drastic as similar changes in uncomplicated adrenal insufficiency (table 1 A). The response to cortical hormone injection is likewise the same in both cases.

An interesting case is that of dog 32 (table 3 E). This animal was given a subminimal maintenance dose of cortical hormone for a few days and this was reflected by a decrease in blood pressure, and an increase in pulse rate and urea nitrogen at the time he was used in the experiment. However, from external observations, he appeared to be normal at the time of injection of 50 cc. of glucose per kgm. In less than one hour he was prostrate and died when a blood sample was drawn at the end of a two hour and ten minute period. The serum sodium level was reduced somewhat below that observed in an animal receiving adequate maintenance hormone, subjected to the same quantity of glucose over a period of three hours (table 3 D).

It is obvious from these experiments that when the serum sodium and chloride concentrations of an adrenalectomized dog are lowered from normal levels, either by glucose injection or trauma, to levels as low as those in true adrenal insufficiency, hemoconcentration and circulatory failure result. Blood dilution does not occur in the absence of cortical hormone, even when the serum sodium and chloride concentrations are only moderately diminished. Following hormone injection, progressive compensation by blood dilution occurs while the serum sodium and chloride levels may have risen only slightly above the lowest levels. A normal blood pressure is invariably attained before the normal electrolyte pattern is established.

Silvette and Britton (1935) report that in the normal cat the tissue and serum sodium and chloride can be reduced to levels much lower than those observed in adrenal insufficiency without deleterious effects.

By injecting a large quantity of glucose intraperitoneally (approximately 100 per cent more than that required for an equal M.-eq. electrolyte reduction in the healthy, vigorous, adrenalectomized dog), the serum sodium and chloride may be lowered in the animal with intact adrenals to levels similar to those of adrenal insufficiency. Nevertheless, the circulation is not so seriously impaired as evidenced by the arterial pressure and general symptoms. These animals spontaneously recover. The

question arises as to why there should be such a marked difference in these respects between the intact and the adrenalectomized dog when the serum sodium and chloride levels have been reduced to similarly low levels. The only important variable between the two types of animal is the presence of adrenal cortical tissue in the intact dog. In our opinion, the presence of such tissue is the answer to this question, for when the hormone is present in adequate amounts blood dilution occurs even at extremely low serum sodium and chloride concentration. This fact can be easily demonstrated by administering cortical hormone to adrenalectomized dogs: 1, in collapse from traumatic shock; 2, verging on death from adrenal insufficiency, and 3, prostrate as a result of intraperitoneal glucose injections. The adrenalectomized dog subjected to such treatment succumbs unless injected with hormone.

SUMMARY AND CONCLUSIONS

1. Mild muscle trauma to healthy vigorous adrenalectomized dogs induces a rapid (6 to 12 hr.) decrease in serum sodium and chloride to concentrations similar to those characteristic of uncomplicated adrenal insufficiency (approximately 6 to 12 days after withholding hormone). The circulatory collapse, as evidenced by blood pressure and symptoms, associated with these electrolyte and fluid changes are likewise comparable.

2. The normal dog subjected to severe trauma to both hind legs shows only a slight reduction in serum sodium and chloride even when in extreme secondary shock. The circulatory failure is due primarily to low blood volume induced by extensive loss of whole blood and plasma into the severely injured areas.

3. The adrenalectomized dog loses a negligible amount of fluid into the slightly injured tissue. The rapid hemoconcentration, low blood pressure and death are due to a generalized shift of fluid and electrolytes from blood stream to the tissues and interstitial tissue spaces, and failure of the diluting mechanism owing to absence of the adrenal cortex. Following cortical hormone injection the normal internal electrolyte and fluid balance is reëstablished.

4. A decrease in serum sodium and chloride is associated with shock resulting from the single stage bilateral extirpation of the adrenals in this species.

5. The adrenalectomized dog although in equally good physiological condition is far more susceptible to the decrease in serum sodium and chloride and associated disturbance in fluid balance following intraperitoneal injections of isotonic glucose than is the animal with intact adrenals.

6. When these serum electrolytes are reduced by glucose in the adrenalectomized dog to levels similar to those of uncomplicated adrenal insufficiency the circulatory phenomena and symptoms are comparable in both

cases. The adrenalectomized dog invariably succumbs to such abnormal electrolyte and fluid shifts induced by glucose administration unless large doses of cortical hormone are injected.

7. The quantity of glucose must be approximately doubled to produce an equivalent diminution in serum sodium and chloride in the animal with intact adrenals. Despite the electrolyte and fluid disturbances, the circulatory changes and symptoms are not nearly so severe as those occurring in the adrenalectomized dog lacking hormone, and spontaneous recovery is invariable.

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A COMPARISON OF THE INFLUENCE OF FASTING UPON THE TOLERANCE TO GLUCOSE AND GALACTOSE

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There has been an accumulation of evidence which indicates that the metabolism of galactose may be considerably different from that of glucose. The ketolytic and nitrogen sparing action of galactose in fasting humans and in those receiving a ketogenic diet was observed by Deuel, Gulick and Butts (1) to be superior to that of glucose. Galactose is a relatively poor glycogen former when fed under the same outward conditions as glucose (2) probably because, at least in rats and dogs, it is lost so rapidly by the organism. However galactose formed glycogen is held much more tenaciously by the organism (3). Galactose unlike glucose is not effective in insulin shock (4) and cannot be used by liverless animals (5). Some investigators (4, 6) suggest the utilization of galactose by the diabetic organism but there is considerable evidence (7, 8) against this. Fasting, as is well known, reduces the tolerance for glucose in the normal subject (9, 10). There is suggestive evidence (11) that in the rabbit fasting is without any influence upon galactose tolerance. An investigation has been made of this point.

A typical result is presented here. Three adult male albino rabbits weighing 4.3, 4.4 and 4.3 kgm. respectively were given intravenous sugar tolerance tests to glucose and galactose, the curves for each sugar being determined both when they were fully fed and after a fasting period of 3 days. In every case the sugar was given in 50 per cent solution and the dose of sugar was always 9 grams. A preliminary blood specimen was taken and the sugar solution then given in the marginal ear vein. Four minutes were required for the injection and blood samples were taken 10, 30, 60, 90, 120, 180 and 240 minutes after the injection was completed. All blood specimens were taken by heart puncture and the proteins removed with Somogyi's (12) zinc hydroxide reagent. Somogyi's modification (13) of the Shaffer-Hartman method was used for the sugar determinations. The amount of sugar lost in the urine was determined in the galactose experiments. All of the results are expressed in terms of glucose.

The data presented in figure 1 demonstrate very clearly the influence of fasting upon the tolerance to glucose. With galactose on the other hand

there is no demonstrable influence of fasting. The blood sugar curves following galactose after full feeding and fasting are practically the same. This can hardly be due to differences in the amount of sugar lost in the urine. After feeding, an average of 2.84 grams of galactose was lost per rabbit and after fasting an average of 3.26 grams.

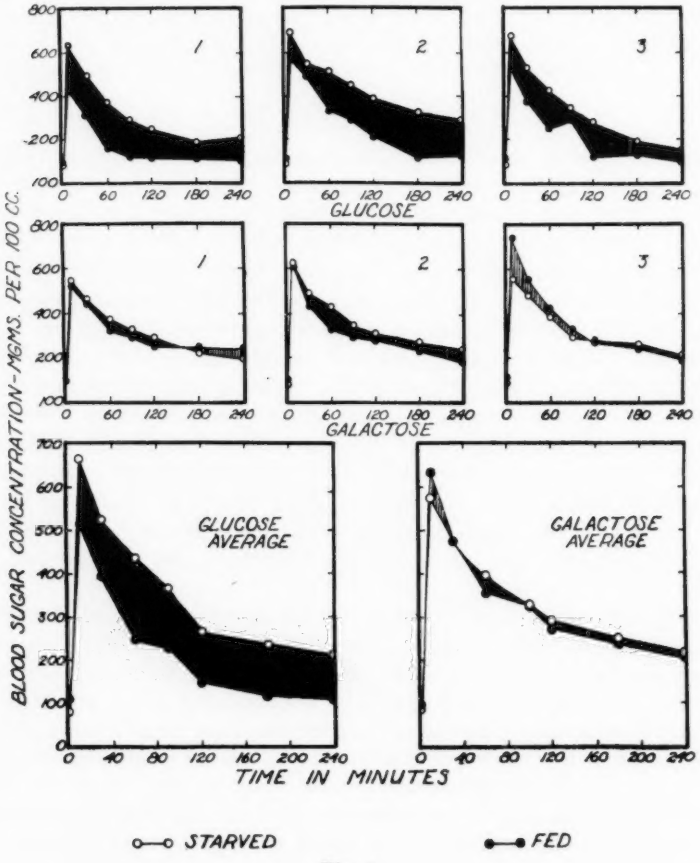

Fig. 1

It is generally accepted that the higher utilization rate or lower blood sugar curve following a second dose of glucose (14) has essentially the same basis as the higher sugar tolerance after feeding in comparison with fasting (9). With this view our findings are supported by the experiments of Sawada (15). He [found [that" the intravenous injection of glucose in

rabbits was followed by a hypoglycemia after the hyperglycemia while after the injection of galactose the hypoglycemia phase was absent. A second injection of the same sugar 2 hours after the first injection was found to give a flatter blood-sugar curve in the case of glucose but not in that of galactose.

The experiments we have given an example of, and those of Sawada were carried out with intravenous galactose injections. The possibility of a difference in the results of intravenous and alimentary administration must be considered, particularly in view of the place of the liver (16) in determining the nature of the glucose tolerance curve. Then Nissen (17) has reported that young persons on a low carbohydrate diet had an abnormally high and protracted hyperglycemia following oral or intravenous adminis-

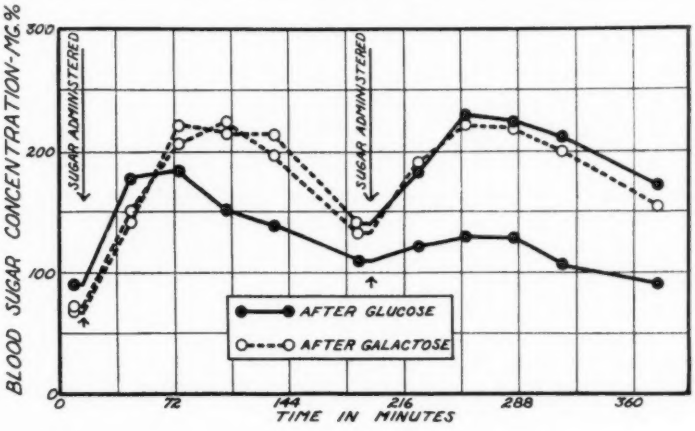

Fig. 2

tration of glucose with similar results following oral but not intravenous administration of galactose. The alimentary hyperglycemia from repeated doses of glucose and galactose was consequently examined.

Figure 2 presents a typical result. These curves were from 3 different male rabbits weighing 3.3, 3.3, and 3.2 kgm. respectively. They had been starved over night. A fasting blood sample was taken and then 3 grams per kilo of the desired sugar in 25 per cent solution administered by stomach tube. Three hours later a second dose of sugar was given. A second dose of glucose (after glucose) gave a very much flatter curve as might be expected (14). The preceding ingestion and absorption of galactose has no influence upon the hyperglycemia due to a second dose of this sugar. Glucose ingestion when preceded by galactose always gave curves similar to the fasting animal. These experiments confirm the results and conclusions drawn from the intravenous administration experiments.

SUMMARY

In contrast with that of glucose the blood sugar curve in the rabbit following intravenous galactose administration is not influenced by fasting.

Unlike that of glucose the alimentary hyperglycemia produced by galactose is uninfluenced by the previous ingestion of glucose nor is the blood sugar curve of a second dose of galactose essentially different from that of the first.

These findings are taken as additional evidence that there may be a fundamental difference in the metabolism of glucose and galactose.

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THE EFFECT OF AN ANTIDROMIC IMPULSE ON THE RESPONSE OF THE MOTONEURONE[1]

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Antidromic shocks have been recently used by the Oxford School (Denny Brown, 1928; Eccles, 1931; Eccles and Sherrington 1931; Eccles and Hoff, 1932) to analyse the process of excitation of the spinal motoneurones by stimuli delivered across a reflex arc. The interpretation of the experimental results was largely based upon the assumption that after arrival of the antidromic impulse the motoneurones enter into a period of refractoriness (absolute for no longer than 2.4σ and relative for no longer than 14σ; Eccles, 1931). At the time the experiments were being carried out no other explanation seemed possible, because the axones were not known to be partially refractory for longer than 5σ (Eccles, 1931, p. 568). Later, however, evidence has been forthcoming which changes the theoretical basis upon which the Oxford School based the discussion of the experimental findings. On one hand the concept of recovery of nerve is in process of evolution (Gasser, 1935; Graham, 1933, 1935) and on the other hand the absolute refractoriness of the neurone, measured by more direct methods has been found to be no longer than that of the axon (Lorente de Nó, 1935b).

It seemed therefore advisable to analyze the effect of antidromic impulses by means of a preparation in which the motoneurones receive stronger stimuli than those produced across an ordinary reflex arc.

TECHNIQUE. The preparation used (rabbit) has been described in a recent paper (Lorente de Nó, 1935a). By means of stimulating electrodes placed on the floor of the fourth ventricle shocks were delivered to the pathways ending on the motoneurones of the third nerve and the action potentials developed by the internal rectus muscle were recorded. The shocks were produced by the double Thyratron stimulator (Schmitt and Schmitt, 1932), mentioned in a previous paper (Lorente de Nó, 1935b).

Antidromic shocks produced by a similar stimulator were delivered to the oculomotor nerve in the base of the skull shortly after leaving the midbrain. The diagrams in figures 1, 2 and 4 show the position of the electrodes

[1] The work reported in this paper has been aided by a grant from the Rockefeller Foundation.

and the paths for the stimuli in each experiment. For the purpose of the present study in which the normal rate of recovery of a nerve had to be determined, it was of paramount importance to keep the nerve as nearly as possible in a normal condition. Therefore the nerve was not prepared; shielded electrodes were introduced through the thalamus until their free points were obliquely placed across the oculomotor nerve. If only the effect of motor shocks had to be examined the third nucleus was destroyed, but if responses of the motoneurones had to be obtained the thalamus and midbrain were not disturbed. But at any rate, in no case was the blood supply of the nerve disturbed, nor of course was its temperature changed. These precautions perhaps account for the much longer recovery periods observed. But even with those precautions a deterioration of the nerve after prolonged stimulation was detected, which may be explained by polarization at the site of stimulation.

The internal rectus muscle was prepared with utmost care not to disturb its blood supply and was not exposed until by means of a suitable vaporizer the temperature of the surrounding air was raised to 37°C. The distal end of the muscle was connected with a silk thread to an isometric myograph and submitted to an initial tension no larger than 1 or 2 grams. The tension developed by the maximal twitch was found to remain constant within very narrow limits during the whole experiment (several hours).

The potentials were recorded with the amplifier and cathode ray oscillograph described in another paper (Blossom and Lorente de Nó, 1934). Two silver- silver chloride hooks applied on the muscle were used as recording electrodes. The ground electrode was placed on the belly of the muscle about 10 mm. from the end, the grid electrode on the killed end of the muscle.

RESULTS. *The recovery of the nerve muscle preparation.* This has been measured in terms of the height of the response to a second (testing) shock weaker than the first or conditioning shock. The height of the crest of the second response has been measured with fair accuracy by superposing the two films obtained with the conditioning shock alone and with both shocks and subtracting the former response from the latter.

The curves obtained by plotting height of conditioned response against interval between shocks (fig. 1) have a different significance when the shocks delivered to the nerve are maximal than when they are weak. If both shocks are maximal the shape of the curve is determined by the statistical distribution of the absolutely refractory periods of the nerve fibres and the size of the potential developed by the partially refractory muscle fibres. But if the second (testing) shock is weak the plotted curve is a fair indicator of the temporal course of the late recovery of the nerve fibres.

Curve *1* and *2* in figure 1 were obtained with supermaximal shocks, so

that the nerve fibres necessarily gave a second response as soon as they recovered from absolute refractoriness. The earliest second response of muscle was obtained at a stimulus interval of 0.52 or 0.60σ. These values are indeed unexpectedly low; they are lower than those found in a previous

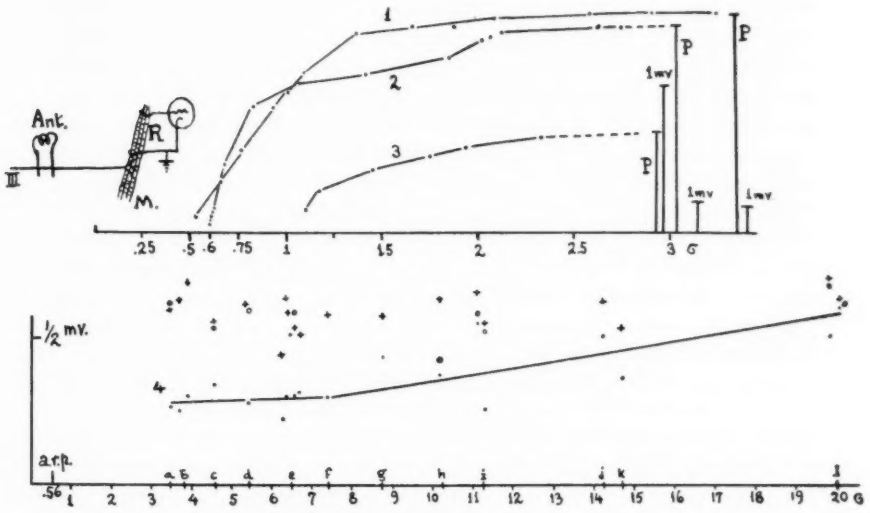

Fig. 1. Curves obtained by plotting the height of the crest of the second response of the nerve muscle preparation (ordinates) against the interval between stimuli in σ (abscissae). The stimulating electrodes (*Ant.*) were placed across the oculomotor nerve (III), the recording electrodes (*R*) on the internal rectus muscle (*M*). *P* is the potential developed by the response to the unconditioned second shock; scale (1 millivolt) at side.

1 and 2. Both shocks supermaximal (*1*, expt. 22/1/35; *2*, expt. 17/1/35).

3. The second shock submaximal (expt. 20/11/34).

4. Both shocks very near threshold, but the second one slightly smaller (expt. 23/1/35). The points of the curve were obtained at the intervals marked by the letters *a* to *l*. No second response was observed at an interval of less than 3.5 σ. At each interval several films were taken, of both conditioned and unconditioned second response. The crosses mark the height of the response to the first shock, the circles the response to the unconditioned second shock and the dots the responses to the second shock preceded by the conditioning one. *a.r.p.*—absolute refractory period measured with two supermaximal shocks.

investigation (Lorente de Nó, 1935b), because the technique has been improved and the muscle was kept in better condition; but they are still higher than the absolutely refractory period of the motor fibres themselves. Recent work of Gasser (1935) has shown that *A* nerve fibres may follow a stimulus frequency of 2000 shocks per second and therefore have to have

an absolutely refractory period less than 0.5σ. Therefore the new value (0.52 to 0.60σ) of the absolutely refractory period of the nerve muscle preparation does not change the classical belief that the muscle fibre, even when stimulated through the end plate, may not follow as high an impulse frequency as its axon, although it comes very close to it.

As already stated the shape of curves *1* and *2* in figure 1 can not be easily analyzed because it is determined both by the statistical distribution of absolutely refractory periods in the nerve and the recovery of the muscle action potential; nevertheless it can be concluded from those curves that the recovery of the muscle action potential is complete 2.5σ after the first response and therefore if a deficit in potential is found later, it has to be attributed to the failure of nerve fibres to respond to the second shock.

Thus, when the second shock is weakened the shape of the curve is primarily determined by the relative refractoriness of the nerve fibres. Curve *3* in figure 1 illustrates an experiment in which the testing shock produced an unconditioned response of about $\frac{1}{3}$ of the maximal motor twitch. The earliest second response was obtained at an interval between shocks no shorter than 1.2σ; the recovery was complete at about 2.5σ.

Using still weaker testing shocks, which do not begin to be effective until 1.5 or 2σ after the conditioning one it has been customary to find depressed second responses until no less than 8 or 9σ after the first shock. Since at that stimulus interval the muscle fibres were developing full-sized potentials it had to be concluded that some nerve fibres failed to respond. But it has been also consistently found that the relatively refractory period thus measured diminished during the experiment, dropping nearly to the figure (5σ) mentioned by Eccles (1931).

Therefore it has been tried to determine the relatively refractory period at the very beginning of the experiment, without previous stimulation of the nerve. The result of a typical experiment is illustrated by curve *4* in figure 1. The shocks used were just above threshold and therefore stimulated only a few of the most irritable fibres. The crest of the action potential was variable in height within rather narrow limits, undoubtedly because the nerve fibres were showing the spontaneous changes in excitability mentioned by Blair and Erlanger (1933). Under such conditions the deter nination of the end of refractoriness had to be more or less statistical.

The experiment was carried out as follows: Beginning at an interval between stimuli of 8σ, at which the second response was still very much depressed, the testing shock was placed at progressively increasing times from the conditioning one, and at each distance several—at least 6—alternative determinations were made of the height of the unconditioned and of the conditioned second response; the positions of the crests were marked with dots on the face of the cathode ray oscillograph. Thus, two

series of groups of dots were obtained which did not coincide until the interval between shocks became as long as 20σ. Before that interval was reached the group of dots of the conditioned response were found consistently below those of the unconditioned one. Immediately afterwards the oscillograph was cleaned and several films were taken which have been used in the construction of curve *4* in figure 1. No repetitive response was observed at any stimulus interval less than 3.5σ and at 7.5σ interval the conditioned response still was only one-half of the unconditioned one.

In two other similar experiments the relatively refractory period was found to last for 15 and 17σ. Therefore, there can be no doubt that the recovery of nerve fibres is much slower than previously assumed.[2]

In the experiment illustrated in figure 1, *4*, one hour later, when the nerve had been stimulated several hundred times the threshold of stimulation became 11 per cent higher and the relatively refractory period dropped first to 9 and somewhat later to 7.5σ. The absolutely refractory period measured with two supermaximal shocks remained constant at the ordinary figure of 0.56σ.

The refractoriness of the motoneurone-nerve-muscle preparation created by a maximal shock delivered to the motor nerve (antidromic shock). It has been demonstrated in a previous paper (Lorente de Nó, 1935b) that the motoneurone when excited across synapses, if any, has an absolutely refractory period which can not be longer than that of the axon. Therefore it could be predicted that an antidromic shock would produce a refractoriness of the motoneurone-nerve-muscle preparation no longer than the refractoriness found in the nerve-muscle preparation. The experimental results have fully confirmed this prediction.

Figure 2 contains several curves constructed by plotting the height of the response elicited across the motoneurones against the interval in sigmas between the antidromic and the testing floor shocks. The potential developed by the maximal antidromic shock is indicated on the axis of the ordinates.

In obtaining curve *1* a maximal stimulus at the floor of the fourth ventricle was used. The response, which of course was being obtained across

[2] The extraordinarily long relatively refractory period (15 to 20σ) mentioned in text, can not correspond to the classical one; it must rather include the period of late subnormality described by H. T. Graham (1933, 1935). Gasser (1935) finds that subnormality develops in normal nerves when repetitive stimulation has created a positive after potential. In my own experiments the stimulation of the nerve was repeated once every one and a half second, so that the conditions for summation of positive after potentials were not present. Therefore it has to be concluded that Graham's period of subnormality may be developed in normal nerves after a single stimulus. However, I think it advisable to use the old denomination "relatively refractory period" for the whole interval of time during which the second response shows a deficit, until a new terminology is agreed upon.

the motoneurones, developed about $\frac{3}{5}$ of the potential of the maximal motor twitch. The earliest second response after the antidromic shock was obtained at 1σ interval; the recovery progressed very fast up to about 2σ interval and then slower; it became complete at about 13 or 15σ.

In obtaining curve *2* the stimulus at the floor of the fourth ventricle was reduced, so that the unconditioned response dropped to about $\frac{1}{3}$ of the

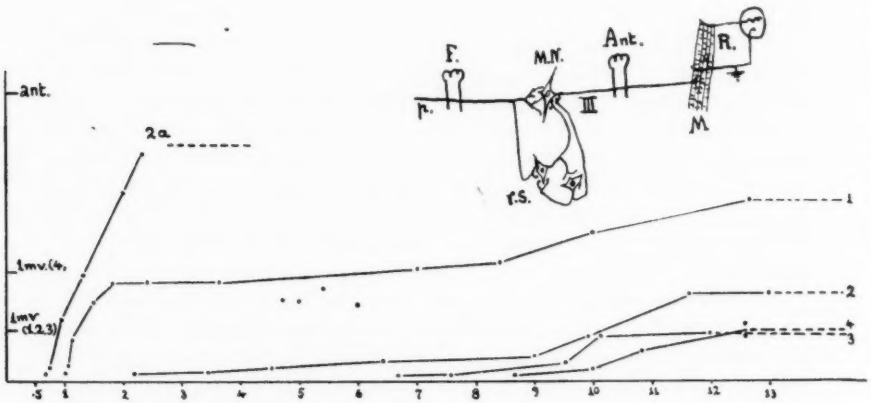

Fig. 2. Stimulating electrodes *Ant.* and *F.* on the oculomotor nerve (III) and on the floor of the fourth ventricle, on paths *p*, which end of the motoneurone *M N* and on reticular cells *r.s.* The responses illustrated by curves *1*, *2*, *3* and *4* were primarily due to the impulses set up in paths *p*, but the responses illustrated by curve *2a* were facilitated by a previous floor shock which although subliminal for the motoneurones excited *r.s.* cells. Recording electrodes *R* on the internal rectus muscle *M*.

The interval between the antidromic and the floor shock is given in abscissae in σ. The height of the response to the floor shock is given in ordinates (scale, 1 millivolt, at left). The potential developed by the antidromic response (maximal motor twitch) is given in the axis of the ordinates (ant.). The horizontal broken lines show the potential developed by the responses to the floor shocks alone. The antidromic shock was produced by discharge of 0.13μf condensor; potentiometer setting 0.15. The floor shocks were produced by discharge of a 0.14μf condensor, potentiometer settings 0,3; 0,17; 0,06; 0,05. In obtaining curve $2a$ two floor shocks were used at 0.6σ interval, the first produced by discharge of a 0.06μf condensor and the second by discharge of 0.14μf one; potentiometer setting 0.17. (expt. 9/1/35).

maximal motor twitch. Accordingly, the earliest second response was observed at about 2.2σ interval; the recovery was at first very slow; it increased rapidly after about 9σ and was complete at about 12σ.

In obtaining curve *3* the floor shock was still further reduced so that it set up a response of only about $\frac{1}{7}$ of the motor twitch. The earliest second response was not observed until the interval between shocks was as long as 6.7σ. The recovery was complete again at 13 to 14σ.

Finally in obtaining curve *4* a floor shock just above threshold was used, which elicited a response no more than $\frac{1}{10}$ to $\frac{1}{11}$ of the maximal motor twitch. The earliest second response was observed at 8.7σ interval; the recovery was complete at about 13 to 15σ.

Since even in the case of curve *1* the stimulus delivered to the motoneurones was relatively weak it was tried to increase it by facilitation, and the same floor shock as in curve *2* was used again, but preceded at 0.6σ interval by another facilitating subthreshold floor shock. The response thus obtained was considerably larger, almost 0.8 of the maximal motor twitch.

The earliest response after the antidromic one was obtained at an interval of 0.56 to 0.6σ between the antidromic and the second floor shock, and the recovery was complete at an interval of no more than 2.25σ (curve *2a*).

Immediately afterwards the response to two supermaximal antidromic (i.e., motor) shocks was recorded and the curve of recovery of the potential of the second response was found to be practically identical with curve *2a* in figure 2, which also is practically identical with curve *2* in figure 1.

On the other hand the similarity between curve *1* in figure 2 and *3* in figure 1 and between *2*, *3* and *4* in figure 2 and *4* in figure 1 is very patent. It indicates that the process of recovery in both cases is very much the same and therefore the preparation motoneurone-nerve-muscle has the same refractoriness as the ordinary nerve-muscle preparation.

The fact that the curves *2a*, *1*, *2*, *3* and *4* in figure 2 start the later the weaker is the stimulating shock, in other words, that the "absolutely" refractory period of the preparation depends on the strength of the stimulus at the floor of the fourth ventricle seems to have only one explanation. It has been shown in previous paragraphs that the fibres of the oculomotor nerve after conducting an impulse become partially refractory during 15 to 20σ, the refractoriness decreasing steadily after its start. The fact now that the motoneurones when weakly stimulated across synapses begin to overcome that refractoriness later than when strongly stimulated indicates that the change induced in them by the stimuli arriving to their synapses may have different strengths. In other words, the intensity of the excitatory process in the neurone can be measured by its ability to set up an impulse in the partially refractory axon. Since by varying the strength of the shock delivered at the floor of the fourth ventricle only the number of stimulated fibres ending upon the motoneurones is varied, it may be concluded that the degree of excitation of the motoneurones depends on the number of activated synapses.

The excitatory process of the motoneurones may attain all degrees, from maximal, when it is able to set up an impulse in an axon immediately after the absolutely refractory period, to threshold-value, when it does not start an impulse until the very end of the partially refractory period.

The dependence of the strength of the excitatory process on the number of activated synapses explains a noteworthy difference between curves *3* and *4* in figure 1 and curves *1*, *2*, *3* and *4* in figure 2. The curves obtained from the ordinary nerve muscle preparation never have sudden steps and as a rule they end the later, the weaker the testing shock. But when the excitation takes place across the motoneurones the curves have definite steps with almost horizontal stretches between each two of them. On the other hand it is often found that in spite of wide variations in the intensity of the stimulus, they may become horizontal nearly at the same interval between shocks (curves *2*, *3* and *4*, fig. 2).

This is easy to understand. The curves obtained with the nerve muscle preparation, when the testing shock is weak really represent the statistical distribution of electrical thresholds (or recoveries) in the nerve. In the case of the motoneurone-nerve-muscle preparation the shape of the curve is determined by two factors: the statistical distribution of thresholds in the motor axons and the statistical distribution of synapses on the motoneurones. The latter is dependent on the branching of fibres within the

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Fig. 3. Stimulating and recording electrodes as in figure 2. The records show the lack of effect of an antidromic impulse on facilitation. Records *5* to *11* have been used in constructing table 1, and records *14–20* in constructing curves *1*, *2* and *3* in figure 4.

1. Timing film ($1/2\sigma$) for records *2* to *11*. 2. Response to a maximal antidromic (motor) shock. 3. Response to two maximal antidromic shocks at 0.72σ interval; the second shock elicits one of the earliest second responses (absolutely refractory period in that preparation $0{,}60\sigma$). The amplification in records *2* and *3* is slightly less than in records *4* to *11*. 4. Two floor shocks at about 1.3σ interval, the second one giving rise to a greatly facilitated response. Note the difference (0.8σ) in latency in records 2 and 4, which, less conduction time from electrodes *F* to electrodes Ant., measures the synaptic delay in the motoneurones. 5. The antidromic shock followed by the second floor shock, which remains ineffective. 6. Same shocks as in 5 but preceded by the facilitating first floor shock. The response to this shock is obliterated by the antidromic impulses, but the second floor shock elicits a response. Distance between antidromic and second floor shock 0.43σ. In records *7*, *8* and *9* this distance is increased and an increase of the second response results. 10. Two floor shocks at 0.65σ interval. 11. The same floor shocks, but the first one preceded 0.1σ by a maximal antidromic, which obliterates the first floor response but does not prevent a response of some facilitated motoneurones to the second floor shock. 12. Timing film (1σ) for records 14 to 17. 13. Timing film (1σ) for records 18–20. 14. Second floor shock alone. 15. Both floor shocks; the second response is facilitated. 16. A maximal antidromic shock placed between the two floor ones; the response to the first floor shock is obliterated, but the response to the second one is only slightly decreased. 17. The second floor shock preceded only by the antidromic. No response is obtained. 18. Two floor shocks, the second one giving a greatly facilitated response. 19. A maximal antidromic shock followed by the second floor shock. No response to the latter is observed. 20. The antidromic shock is preceded by a facilitating floor shock. The second floor shock produces now a strong response.

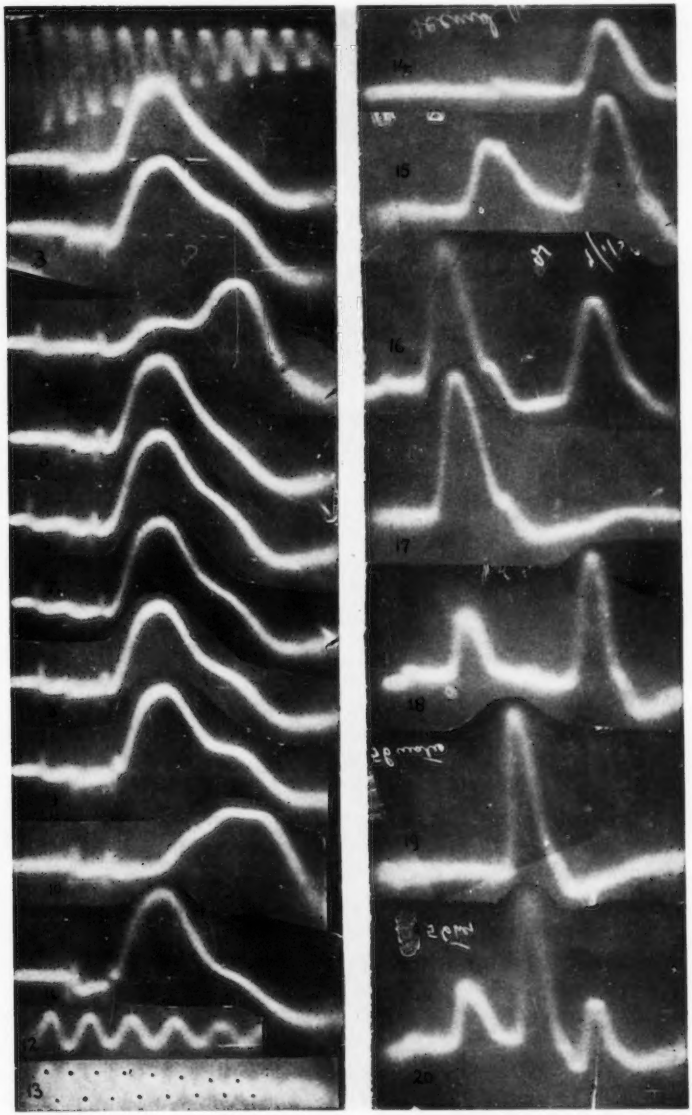

Fig. 3

motor nucleus, and therefore it may happen that following a certain shock at the floor of the fourth ventricle neurones with axons of high electrical threshold have many activated synapses while neurones with axons of low electrical threshold have only a few. For the floor shock both kinds of motor axons will have the same threshold.

The effect of an antidromic shock on facilitation. When a shock delivered through electrodes placed on the floor of the fourth ventricle is preceded at a suitable interval by another shock through the same electrodes a facilitation of the response to the second shock is produced, which may be demonstrated as well when both shocks are subliminal as when both are liminal. The temporal course of facilitation varied from preparation to preparation but remained constant in its general traits. In many cases it lasted sufficiently to make it possible to intercalate an antidromic shock between the two floor shocks.

The results of a considerable number of experiments have been absolutely constant. In no case has it been possible to demonstrate that the antidromic impulse had destroyed the facilitating effect of the first floor shock. The modification of the facilitated response was apparently due to the refractoriness created in the motor fibres by the antidromic shock.

Figure 3 reproduces several records and figure 4 contains several typical curves illustrating the recovery of the response to stimuli at the floor of the fourth ventricle after an antidromic shock. Curves *1*, *2* and *3* in figure 4 belong to the same experiment. Two maximal floor shocks were used, each one alone giving rise to a response which developed the potential *P 2*. When facilitated the second shock gave rise to a response with potential *P 1*, almost $\frac{9}{10}$ of the potential *P* of the maximal motor twitch.

Curve *3* illustrates the recovery of the response to the second floor shock when preceded only by the antidromic one. No second response was observed until the interval between shocks was 1.45σ. The second responses increased in size very slowly and were not entirely recovered at an interval between shocks of 7σ. Measured in terms of the refractoriness it was able to overcome, the excitatory process of the motoneurones created by the unconditioned floor shock was rather weak.

Curve *2* illustrates the response to the second floor shock when preceded by the facilitating floor one and the antidromic. The distance between both floor shocks (marked by arrow 2) was 4.4σ, so that the antidromic shock was always placed between them.

The earliest response to the second floor shock was obtained at 0.6σ interval, i.e., almost immediately after the nerve fibres had recovered from the absolute refractoriness created by the antidromic impulse. Therefore the antidromic shock had not destroyed the facilitation created by the first floor shock, which raised the excitatory process created by the second floor shock to maximal value. The recovery of the facilitated response

proceeded then very fast and became complete at about 5σ. Curve *2* is at every shock interval considerably higher than curve *3*.

In obtaining curve *1* the distance between both floor shocks was reduced to 2.45σ. The results were again the same. The antidromic schock never destroyed the facilitating effect of the first floor shock.

In another typical experiment, the distance between floor shocks was reduced to 0.8σ. The antidromic shock at the beginning was placed between and later preceding both floor shocks. Facilitation was present at every interval between shocks and the curve plotted as above did not show any break when the antidromic shock was simultaneous with the

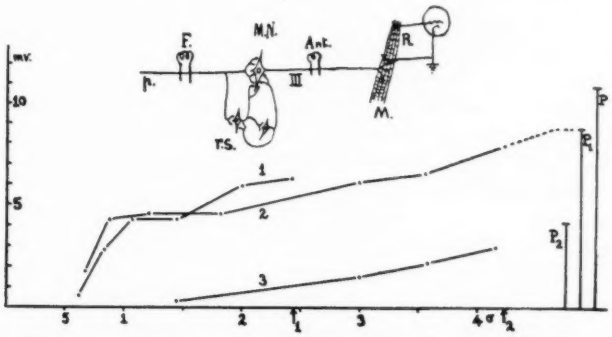

Fig. 4. Stimulating and recording electrodes as in figure 2. The experiment was arranged as indicated by records 14–20 in figure 3. The curves are a plot of the height of the response to the second floor shock when preceded only by the maximal antidromic (3) or by facilitating floor and a maximal antidromic (1, 2) (expt. 7/1/35). The interval between the antidromic and the second floor shock is given in abscissae. Arrows 1 and 2 indicate the constant interval between the two floor shocks in curves 1 and 2 respectively, so that the interval between the first floor shock and the antidromic has to be read from the arrow toward the origin.

P, potential of the antidromic response; *P2*, potential of the response to the second floor shock alone; *P1*, potential of the response to the facilitated second floor shock.

facilitating floor one. Undoubtedly, even in that case the facilitation remained unaffected by the antidromic impulse.

The same results have been obtained in all the other experiments (five) in which the facilitation was strong enough to raise the excitatory process to maximal value in a number of motoneurones. In one experiment in which a large number of motoneurones were excited maximally by the facilitated second floor shock, it was tried to determine the shortest possible interval between antidromic and second floor shocks, at which a repetitive response appeared. Several of the values are given in table 1. Figure 3 contains a few of the records obtained.

It is evident from the previous experiments and from this table that the

position of the antidromic shock in relation to the facilitating floor one is of no consequence; the appearance of a second response depends solely on two factors: *a*, the distance between floor shocks, which determines the amount of facilitation and therefore the strength of the excitatory process of motoneurones, and *b*, the distance between the antidromic shock and the testing floor one, which determines the degree of recovery of the axons after the antidromic impulse.

TABLE 1

Intervals in σ between a conditioning floor shock (F1), a maximal antidromic shock (Ant.) and a testing floor shock (F2) at which a second response due to F2 was observed

If F1 was omitted, F2 remained ineffective (expt. 11/1/35). Values ±0.02 σ.

F1	0.62 σ Ant.	0.62 σ F2
F1	0.51 Ant.	0.85 F2
F1	0.80 Ant.	0.43 F2
F1	0.70 Ant.	0.60 F2
F1	0.13 Ant.	0.68 F2
F1	0.00 Ant.	0.65 F2
Ant.	0.10 F1	0.65 F2

DISCUSSION. The main problem to be considered now is whether or not the antidromic impulse enters into and modifies the state of the body and dendrites of the motoneurones. Sherrington (1900, p. 798) and Eccles and Sherrington (1931) take it for granted that the antidromic impulse passes into the neurone body and dendrites and is first detained at the synapses. But recently Forbes (1934) argues that the antidromic impulse may be stopped at the axon hillock and therefore may not affect the motoneurone at all.

It is true that the antidromic impulse does not seem to start a new potential in the cell body (Gasser and Graham, 1932; Hughes and Gasser, 1934), but of course a qualitatively new potential could hardly be expected. On the other hand it is difficult to visualize how an antidromic impulse could be stopped at the axon hillock, where the axonal and the cell membranes are continuous; it is the more difficult to understand as there are cases in which it is positively known that the impulses pass through the body of the nerve cells. For instance, the ganglion cells of Scarpa and Corti are bipolar and therefore the impulses originated in the internal ear have to pass through their bodies. Certainly there are some histological differences between the ganglion cells of the eighth nerve and the motoneurones but whether those differences might account for such a radical change in functional activity is so doubtful, that only an extremely well

controlled evidence would justify the assumption that the antidromic impulse does not penetrate into the motoneurone. After all the body and the dendrites of the neurone have to be considered only as a specialized part of the axon, adapted to the function of receiving the excitatory impulses. That the body contains the nucleus and certain other structures does not oppose this assumption because often enough the body of the nerve cell lies clearly outside the path of the impulses (Cajal, 1909, 1911, 1934).

On the basis of this assumption it is easy to understand that an antidromic impulse does not produce any other result than to make the cell refractory and does not affect facilitation because the facilitating and in general the excitatory mechanisms are located outside the motoneurone.

In the presentation of the experimental results no reference has been made to the synaptic delay. The existence of a synaptic delay signifies that impulses arrived at the synapsis set up a new impulse in the axon of the motoneurone after a certain period of time, and it is very noteworthy that the synaptic delay is not lengthened after arrival of an antidromic impulse. At first the refractoriness created by the latter prevents the setting up of a new impulse, but when the earliest response is obtained, it is set up after the customary synaptic delay.

The comparison of records *2* and *4* or *10* in figure 3 shows that the synaptic delay in that preparation measured about 0.60 to 0.65σ, because the distance between the *F* and the *Ant.* electrodes (fig. 2) was about 20 mm., and the comparison of record *4* with records *6*, *7* and *8* demonstrates that the synaptic delay was practically unchanged even in the earliest second response of the motoneurones, because the second crest in records *6*, *7* and *8* is found very nearly in the same position as in record *4*. As a matter of fact, it seems to have moved forwards. Since as record *3* shows, the early second responses in the nerve-muscle preparation are considerably delayed one should be inclined to think that after an antidromic impulse the synaptic delay of the motoneurones is shortened; at any rate it is not lengthened.

The shortest possible interval between an antidromic and an effective floor shock has been determined to be 0.43σ; adding to it the synaptic delay (0.60 to 0.65σ) it results that the floor shock has set the earliest second impulse in the axon about 1.15σ after the antidromic one, i.e., about 0.5σ later than a maximal induction shock delivered to the nerve (see fig. 1, *1* and *2*). This explains why the earliest second crest in the motoneurone-nerve-muscle preparation (records *5*, *6*, *7*) appears somewhat later than in the ordinary nerve muscle preparation. However it has to be mentioned that in other experiments in which on the one hand the stimuli created by the floor shocks were stronger (because they produced, without facilitation, maximal motor twitches), and on the other hand both impulses had to pass

through the motoneurones the second responses appeared as early as in the ordinary nerve muscle preparation (compare fig. 3, records *2* to *11*, with figure 2 in Lorente de Nó, 1935b).[3]

Under such conditions it is evident that the mentioned shortest possible interval of 0.43σ measures the upper limit of the absolutely refractory period of the dendrites and cell body for the particular strength of stimuli used in that experiment. As soon as the cell body (i.e., thickened or receptive end of the axon) recovered from absolute refractoriness the impulses which arrived at the snyapses were able to set up an impulse in the axon, after the ordinary synaptic delay. And that figure 0.43σ also is the upper limit of the time during which the excitatory process at the synapsis remains at full strength, because if it should remain at full strength for a longer period of time the interval between the antidromic and the earliest effective floor shock would be shorter.

With the determination of that low value (0.43σ) for the shortest interval between antidromic and earliest effective floor shock a direct proof is given of unidirectional conduction across the central synapse. The distance between the two stimulating electrodes (*Ant.* and *F*, fig. 2) was about 20 mm. Even assuming that the antidromic impulse had been conducted through the third nerve and after crossing the synapse through paths *p* at a speed of 100 m. per second it could not have reached electrodes *F* earlier than 0.2σ after the antidromic shock. Under such conditions it would remain only 0.23 for the absolutely refractory period of the *p* fibres. It is evident then that the antidromic impulse does not cross through the synapses. With these experimental data the question as to the ultimate cause of the dynamic polarization of the neurone (Cajal, 1891, 1900; v. Gehuchten, 1900) is decided in favor of Sherrington's assumption (1900) of a block of the impulse at the synapses.

The concept of the neurone as a nerve fibre provided with a trophic centre and two specialized endings affords satisfactory means of understanding the rôle of the intercellular connections within the nerve centres, as discussed in other papers (Lorente de Nó, 1934) because the thickened part of the axon (body and dendrites) also will have the two types of responses known to exist in the ordinary nerve fibres (Lucas, 1917): local responses capable of summation and conducted or all-or-none responses.

In closing these theoretical remarks it has to be mentioned that in early days the existence of "local" summation at certain points of the neurone

[3] The comparison made in a previous paper (Lorente de Nó, 1935*b*, page 287) of the earliest second responses in the nerve-muscle and in the motoneurone-nerve muscle preparation is not entirely correct because with the former (fig. 1 in that paper) only submaximal shocks were used, which of course did not excite the motor fibres immediately after the absolutely refractory period, but some time afterwards. The comparison made in later experiments under identical conditions for both preparations has shown that there is no measurable delay of the second impulse during its passage through the dendrites and body of the neurones.

was postulated by Sherrington (1925, p. 524) and that the interpretation given by Hughes and Gasser (1934) of the potentials found in the spinal cord during activity, clearly is based upon the same concept used above, that the neurone body has the same elemental properties as the axon.

SUMMARY

The effect of an antidromic impulse on the response of the motoneurones has been studied in a preparation in which the motoneurones receive stronger stimuli than through the ordinary reflex arc.

The antidromic shock does not produce any other effect than to make the axon refractory; it does not destroy facilitation.

The refractoriness of the axon, also measured in the ordinary nerve muscle preparation lasts for about 20σ. This long refractoriness is interpreted as being due to the sub-normal period of nerve discovered by H. Graham (1933, 1935) and again found by Gasser (1935).

The ability of the neurone to overcome the depression of the excitability of the axon gives a measure of the strength of the excitatory process created at the synapses.

A direct proof of unidirectional conduction across the central synapse is given.

The neurone is interpreted as a nerve fibre having two specialized endings, the receptor and the effector ones. The trophic centre with the nucleus may be located within the fibre itself and acquire the shape of cell body with dendrites.

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DETERMINATIONS OF REFRACTORY PERIODS IN THE TURTLE HEART

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There is an extensive literature dealing with the responsiveness of cardiac muscle to electrical stimuli and the changes of excitability manifested during the cardiac cycle, under the influence of vagus or sympathetic stimulation, of drugs, and so forth. A critical review of this literature would demand consideration not only of such variables as these mentioned but also of the various experimental conditions involved, such as changes in heart rate and in form of the stimulating shock used. A recent study of changes in electrical excitability changes during the cardiac cycle has been made by Eccles and Hoff (1934). Although they attempted to restrict themselves to the pacemaker of the cat's heart, the curves which they present resemble, in general, those which might have been plotted for other parts of the heart. In considering any series of excitability curves of this sort one should continually bear in mind the fact that changes of excitability may result from a variety of causes and that the changes which appear are in some cases capable of algebraic summation. Therefore conclusions as to the effects of any single procedure, such as vagal stimulation, are valid only if all other conditions, notably heart rate, be maintained constant. Fundamental though this requirement may be it has at times been overlooked. Furthermore, differences in form and duration of the shocks used may result in differences in the results obtained and consequently in the conclusions drawn from the experiments performed.

There have recently been described (Gilson, 1935) certain striking differences in the apparent cyclic changes in excitability in normal and in vagus-inhibited turtle hearts according as to whether the excitability is measured by induction shocks of short duration or by double condenser charges of much longer duration. The difference between the results obtained by the two methods seemed sufficiently striking to warrant reinvestigation of the changes in excitability during the cardiac cycle and also of the changes in form of the excitability curves with changes of rate and with vagus inhibition. Certain results obtained in this investigation are presented in this paper.

Determinations of refractory phases in the turtle ventricle. Determinations of excitability as measured by induction shocks were attempted some years ago using the quiescent, excised turtle ventricle. Stimulation was accomplished by breaking a spring switch which was demonstrably variable in action. The results obtained indicated that following a response of the ventricle there was a period of increasing excitability which lasted over a period of many seconds. Determinations were then attempted by means of a device similar in basic concept to that used by Andrus and Carter (1930). A lead was made from the electrocardiograph amplifier to a calibrated delay mechanism. This involved the discharge of a negative bias on the grid condenser of a gas-filled triode. The resulting activation of this triode caused the closing of a relay switch which in turn allowed the discharge of a condenser through a Thyratron. This Thyratron shock was used as a stimulus directly in a few experiments but in most instances was passed through the primary of a Porter coil and the shock from the secondary coil was used as the stimulus. In either case, control of the shock strength was accomplished by a voltage divider the derived circuit from which included the stimulating electrodes and the tissue. By making connections from the exposed, intact and beating heart to the amplifier, the R- or S- wave of the electrogram could be made to commence the series of events which terminated with the delivery to the heart of a shock of desired strength applied at any desired time. The results obtained with this technic were in general similar to those obtained by Eccles and Hoff. Threshold values or times were not well reproduced, especially after the use of shocks of four or five times threshold for determination of the earlier points on the recovery curve. The forms of the plotted curves varied considerably in different preparations. No definite "super-normal" phases were plotted from our data, but several preparations yielded curves which were quite flat during the latter part of diastole.

Recently in experiments using double condenser charges (fig. 1) as the stimulus, rather different and much more consistent results have been obtained. The stimuli used in most experiments had a time to maximum voltage of 0.018 second. This time constant may be changed through a rather wide range (0.003 sec. to 0.150 sec.) without changing the relative values along the curves plotted if determinations for any given curve are all made with a stimulus of fixed form. However, the absolute voltage values of the threshold stimuli plotted may be significantly altered by change in form of the stimulus. In these experiments, the use of the action potential as a means of activating the stimulating mechanism was discarded since it multiplied the technical difficulties of making determinations and since the use of a manually operated switch yielded results having a degree of accuracy which was sufficient for the present work. As the stimulator was arranged during the course of the experiments reported

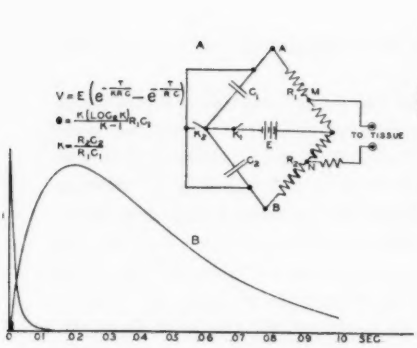

Fig. 1

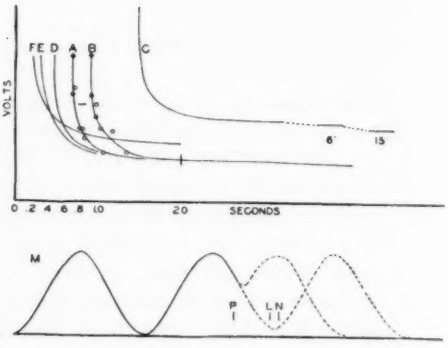

Fig. 2

Fig. 1. *A*. Diagram of circuit used for stimulation by double condenser charges. *B*. Curves showing the form of such a stimulus as compared with the form of a shorter single condenser shock as obtained directly from our Thyratron stimulator and with the approximate duration (not form) of an induction shock.

For the double condenser charge plotted here, R_1 and R_2 were each 55,000 ohms, C_1 was of 0.25 and C_2 of 0.5 mfd. capacity; theta = 0.018 second. The maximum voltage between M and N was 0.0256 volt for every volt applied by the battery, E. For refractory period determinations, the switches were complicated to allow a second shock to be applied through the same network.

Fig. 2. Curve *M*. Diagram of the mechanical response of turtle ventricle indicating the conditions under which curves A and B were obtained. Following two normally activated beats of the ventricle, if S1 were not applied, a normal systole would have commenced at time *N*. Application of S1 approximately at the time *P* elicits an early premature systole. A plot of threshold values of S2 at various times following S1 so placed yields the curve *A*. Measurements of excitability following a systole elicited by S1 placed at time *L* yield data from which *B* was plotted.

Curve *A*. Curve of recovery of excitability following an early premature systole of ventricle of normally beating turtle heart.

Curve *B*. Same but for late premature systole. The curve is extended on a basis of data obtained on the non-rhythmic ventricle and on the ventricle of the heart in standstill under the effect of vagus inhibition (see text).

Curve *C*. Curve of recovery of excitability plotted for excised, quiescent ventricle. Same preparation as for *A* and *B* but ½ hour to 1 hour later. No perfusion.

Curves *D* and *E*. Excitability curves plotted for atrium after late and early premature beats, respectively.

Curve *F*. Excitability curve plotted for late premature beat of atrium under vagal inhibition. The left vagus was stimulated. There was no change in rate of the heart.

To avoid confusing the curves, points actually determined are given only for curves *A* and *B*. About the same number of points having about the same deviation from the smoothed curve was obtained in each case.

Curves *A*, *B* and *C* are plotted directly from recorded data. For purposes of comparison, curves *D*, *E* and *F* have been plotted as if the late diastolic threshold of the uninhibited atrium had been the same as that of the normal ventricle. Actually the threshold determined for the atrium in this experiment was somewhat above that for the ventricle.

The raised threshold for curve *C* is not to be attributed merely to the fact of excision of the ventricle. An excised ventricle may show either an increase or decrease of threshold values depending upon the resultant of a number of factors of which some are physiological and others are physical.

here, the operation of a double-throw, double-pole switch delivered one stimulus (S1) to the heart and commenced the chain of events which resulted in a second stimulus (S2), of desired strength, being delivered at a predetermined time thereafter. S1 caused the systole, following which the course of the recovery of excitability was determined. Since varying the time in the ventricular cycle at which S1 was delivered, significantly changed the course of the plotted curve obtained from the time and S2 data, two recovery curves are given, for example curves A and B of figure 2. Of these curves, A represents the course of recovery after the beat obtained when S1 is thrown in early in diastole. The other curve, B, is obtained when S1 is thrown in late in diastole, thus giving a nearly normal systole for the heart beating at its normal rate. To stimulate early in diastole, it is necessary that S1 be considerably above a minimal stimulating value but the use of this stronger stimulus has been demonstrated not to change the form of a "late" curve from that which would have been obtained with a minimal strength for S1. Another possible source of error was that a time error in placing S1 will make a difference in the position of the corresponding point on the recovery curve determined by the S2 shock. However, it is probable that S1 can be placed with an error of less than 0.1 second and it is a fact that the position of a given strength of S2 just above threshold is reproducible to within 0.03 second and usually to within 0.01 second error when checks are made immediately. Over a longer period, fluctuations of thresholds may change absolute values somewhat though relative values remain without significant change for periods of one to two hours. For a heart beating with an intersystolic interval of about 1.5 seconds, as is the case in most of our experiments, the errors introduced by the methods used are therefore less than the error which could be tolerated at the present stage of our knowledge.

Figure 2 presents curves (*A*, *B* and *C*) plotted from the data of a typical experiment. The brain of the turtle (*Pseudemys elegans*) was pithed, the plastron and front legs were removed, the vagi cut high in the neck, and the pericardial sac was opened widely. The stimulating electrode was a silver, ball-tipped wire, the contact face being flattened and having a diameter of about one millimeter. This was placed lightly against the surface of the heart. For this experiment, the electrode was placed against the ventral surface of the ventricle about one-third of the way from the apex to the basal margin. An indifferent silver electrode was placed in the intestinal mass. Curves *A* and *B* were obtained with the intact, spontaneously beating heart. The temperature was 26.5°C., the intersystolic interval 1.6 second. The strength of S1 (60 volts) is indicated by the short horizontal line. For every volt applied to the bridge (the values recorded) something less than 0.026 volt maximum was applied to the electrodes. The curves show in each case the earliest time at which an S2 stimulus of

voltage as plotted along the ordinates will elicit a response. Curve *A* is plotted from points made with S1 early, curve *B* with S1 late in diastole. Curve *A* is seen to lie below curve *B* for all points plotted. With the beating heart in good condition this is the result which we have always obtained. The amount of separation of the curves given here is somewhat greater than average.

Curve *C* was obtained from use of the excised, non-rhythmic ventricle and determinations were begun about half an hour after completion of determinations for *A* and *B*. It is seen that in comparison with the earlier determinations made on the ventricle of the intact heart, these later determinations show that the thresholds were increased and that the absolutely refractory period was increased. The general form of curve *C* is similar to that of curves *A* and *B*. For threshold determinations with the shorter S1–S2 intervals (up to 2.6 sec.), stimulation was maintained at regular intervals with 10 second pauses between successive S1 stimuli. The use of the non-rhythmic preparation makes possible the demonstration of two facts not to be seen in the beating heart. The first of these is the continued increase in excitability which can be followed almost invariably for 20 seconds and frequently for as much as 30 seconds. In this experiment only two such late points were particularly located, one for a stimulus of 45 volts which was effective after 15 seconds and not after 10 seconds, the other for a stimulus of 48 volts effective after 6 seconds but not after 5 seconds. The positions of these points were not due merely to casual fluctuations in excitability but were in each case checked by repetition of determinations. With respect to the second fact, it has been found that in curves *A* and *B* the curve of recovery from an early premature systole (curve *A*) lies below the recovery curve determined for a late systole (curve *B*). However, determinations made on the non-rhythmic ventricle show that at later times the recovery curves usually cross so that the earlier relationship is reversed. As specifically determined in this experiment, at a time 2.6 seconds after an S1 stimulus, a 52 volt stimulus was effective when the ventricle had had a previous resting interval of 30 seconds but was ineffective when the resting interval had been but 10 seconds. The precise course of the curves in this and the still later portion depends upon a number of factors, the delay in recovery becoming greater for example with a fatigued or anoxic preparation.

Stimulation of the vagus produced no change in ventricular excitability as a primary effect. If vagus inhibition stopped the heart, a systole was followed by a period of rising excitability which continued for many seconds in a manner similar to that seen with the non-rhythmic ventricle. This continued rise of excitability was such that the final threshold values were between 10 per cent and 20 per cent lower than the lowest values obtained on the normally beating ventricle. The apparent increased

excitability is therefore considered as being due merely to the standstill of the heart and not to any primary effect of the vagus upon the turtle ventricle.

Determination of refractory phases in the atrium. The refractory phase determinations made on the turtle atrium yielded curves similar to those plotted for the ventricle (fig. 2, *D* and *E*). The absolutely refractory phase of the atrium is significantly shorter than is that of the ventricle. Difficulties due to stimulation of vagus fibers in the atrium usually did not interfere with normal determinations if several beats were allowed between successive determinations.

Early in the course of this work, an attempt was made to determine changes of excitability in the atrium during periods of vagus inhibition by means of induction shocks. The shortened period of absolute refractoriness reported by Lewis, Drury and Bulger (1921) and by Andrus and Carter (1930) for the partially inhibited atrium was easily demonstrated. However, no consistent change in the late diastolic excitability could be demonstrated in the inhibited atrium which was beating at a rate unchanged from the normal. Referring now only to determinations made with induction shocks applied late in diastole, it may be said that in some preparations vagus stimulation produced no measurable change in excitability, in other cases there was a rise of threshold, in still other cases there was a fall of threshold. In some preparations only the increase was found, in other preparations only the decrease was found and in still others determinations at different times gave at one time an increase and at another time a decrease of threshold. In no case did we measure a change of threshold of over 5 per cent and in only occasional instances was the measured change over 2 per cent.

Since the use of such short induction shocks yielded only such negative or negligible results, we sought for other means of stimulation which might yield results more consistently correlated with the obvious functional depression appearing in the heart during periods of vagus inhibition. Two groups of facts seemed to have significance in this connection. First, there was the statement repeatedly made in the literature and best demonstrated, so far as we are aware, by the data of Ashman and Garrey (1931) that there is a rise of the rheobasic current necessary for stimulation of the vagus-inhibited as compared with the normal atrium. Secondly, there are the relationships presented by Monnier (1934) in his exposition of the concept of pararesonance. Monnier's development of this concept is a logical outgrowth of the discovery by Lapicque (1908) that two double condenser charges of different and suitable forms, respectively, may be used to stimulate selectively the nerve from a toad or the nerve from a frog. Monnier has greatly extended the theoretical and experimental investigation of stimulation by double condenser stimuli. He reports that

for a double condenser stimulus of optimal form for stimulation of a frog nerve (1) the time to maximal voltage of the stimulus is equal to the utilization time for the nerve, (2) that the threshold voltage for a stimulus of this form is only slightly more than the rheobasic voltage for a rectangular constant current, and (3) that the nerve stimulated by such a wave at just threshold voltage produces a response of the nerve which begins at or very close to the crest of the wave. It seemed obviously desirable, therefore, to attempt the use of such stimuli. In preliminary determinations the stimulator used had the values $K = 2$ and *theta* $= 0.018$ second (see fig. 1). The value *theta* $= 0.018$ second was obtained by employing such resistances and condensers as were immediately available in the construction of a stimulator having a theta value approaching the utilization time of 0.02 to 0.025 second previously found by Gilson and Peugnet (1932) for turtle ventricular muscle.

The use of such stimuli to determine excitability in the heart during periods of vagus stimulation which caused no change in rate, revealed a marked and invariable rise above normal of the late diastolic threshold values *if these were determined on parts of the heart showing functional depression as a result of the vagus stimulation.* Whether the differences observed between the results as obtained with induction shocks in the one case and with double condenser charges in the other case are primarily due to shock form differences or to the high voltages necessary for stimulation with the short induction shocks, we are not at present able to say. However, the reproducibility of threshold determinations made with the double condenser stimuli has been so much greater than was formerly possible with induction shock determinations that in recent experiments we have confined ourselves to the use of the former. The results of experiments in which only this type of stimulus was used are reported below in more detail.

If the left vagus is stimulated so as to cause a marked inotropic depression of the atria without change of heart rate, the curve of the recovery of excitability following a contraction is significantly changed from the normal. The absolutely refractory period is shortened and recovery from relative refractoriness is greatly slowed (fig. 2, curve *F*). If moderate vagus stimulation results in a slowed or stopped heart, as may occur with right vagus stimulation, the slowing of the rate results in a lengthening of the absolutely refractory period. Furthermore, a single threshold determination made after a long resting interval may approach or achieve a normal late diastolic value. Thus it is possible, under such conditions, to obtain single point determinations which show no change from normal values. With standstill resulting from more intense vagal stimulation, the delay of recovery is so great that determinations made as much as 30 seconds after a previous beat will still show elevation of threshold. For

atria beating with unchanged rate but showing moderate inotropic depression as a result of vagus stimulation, threshold determinations made late in systole have regularly shown increases of from 50 per cent to 75 per cent. Valid increases of 100 per cent to 200 per cent have probably been observed but in these cases the inotropic depression of the stimulated atrium has been so intense that no visible contraction could be observed, the only sign of response being that at another part of the heart. It is believed that these responses were due to effective stimulation at the electrode followed by conduction in the normal manner to other parts of the heart. However, the use of suitable recording means will be necessary before such findings can be considered as adequately demonstrated.

Discussion. Attempts to determine changes in the functional state of excitability of the heart by means of thresholds to induction shocks have not been satisfactory. The results of such experiments lead one to choose between two possibilities. The first of these is the belief that electrical stimuli do not measure the functional state of excitability. This is the conclusion reached by Eccles and Hoff with regard to the pacemaker of the cat heart. The other possibility is that an induction shock is not an adequate or valid form of stimulus to use in measurements of cardiac excitability. For the present we are inclined to accept the latter alternative. Further experiments may show that this latter alternative is not exclusive of the former. However, we have been struck by the closeness of agreement between the apparent functional state of the heart and the thresholds to double condenser stimuli under the conditions with which we have worked. With respect to the agreement between our experiments and those of former workers, we may point out that there is general agreement of the results in those cases in which the experimental procedures were similar. That our conclusions differ from those generally accepted at the present time is due to the fact that the stimuli which we have employed give different results from those obtained with induction shocks.

If we define, for use in this paper, the word *excitability* as "a reciprocal function of the maximum voltage of a double condenser stimulus such as has been described above and of strength just sufficient to elicit a response at the moment considered," the following synopsis may be presented. For the ventricle of the turtle there is a period of complete inexcitability which probably coincides in time with the period of systole at any given point. Following this period of absolute refractoriness, there follows a period of relative refractoriness during which the excitability rises. In a normal, spontaneously beating heart, the ventricular excitability is still rising at the time of arrival of the next normal impulse. If the data obtained from the excised, quiescent ventricle and from the ventricle of a heart in vagus standstill be considered, the curves obtained with the beating ventricle might be extended. From such an extended curve we

would conclude that, following a systole, the excitability continues to rise for a period of 20 to 30 seconds and reaches a level which is probably 10 per cent to 20 per cent above that attained by the intact ventricle of the beating heart. The period of absolute refractoriness is shorter following an early premature beat than it is following a normal or slightly premature beat. This difference is comparable with the difference to be seen in a rapidly beating as compared with a slowly beating heart under otherwise identical conditions, the rapidly beating heart having a shorter and the slowly beating heart a longer period of systole. The very late part of the recovery curve may show a delayed recovery in the case of the early beat. This last factor does not appear in the curves of the ventricle of a normally beating heart in good condition. It may become significant in a sufficiently depressed heart and may usually be seen in the excised quiescent ventricle.

Concerning the atrium, a somewhat similar statement could be made. The absolutely refractory period is much shorter than is that of the ventricle. Moreover, in the atrium, there may be demonstrated the direct effects of vagus inhibition. Stimulation of the vagus nerve causes a shortening of the absolutely refractory period and a marked slowing of the recovery of excitability following a contraction. It is believed that considerations of functional changes in excitability and conduction should take into account not merely the changes in the absolutely refractory phase but also changes in the time course of the recovery from refractoriness. Whereas a normal heart will conduct an impulse from one chamber to another at a time certainly not much later than the end of the absolutely refractory period, it is obvious that in an inhibited or depressed preparation this is not the case. In such a preparation, it seems probable that time must be allowed for more or less complete recovery from relative refractoriness before conduction across a junctional region will occur. Furthermore, since the absolutely and relatively refractory periods may vary independently one from the other, a measurement of the absolutely refractory period is by no means a valid measure of the course of the relatively refractory period.

SUMMARY

1. Double condenser stimuli have been used for determination of the refractory period curves of the turtle ventricle and atrium.

2. Although the time to maximum of the stimuli used was in most experiments 0.018 second, this time may be varied over a considerable range without changing the form of the excitability curves plotted. Absolute values may be changed by such change of shock form.

3. For either ventricle or atrium of the normally beating heart, excitability as determined by such stimuli begins to rise at the end of the period

of absolute refractoriness, continues to rise without intermission, and is still rising at the time of the beginning of the next normal beat.

4. Under vagus inhibition there is a shortening of the absolutely refractory phase of the atrium. However, the relatively refractory period is prolonged and recovery to normal threshold level may be delayed indefinitely.

5. Vagus stimulation was not in any experiment observed to produce any direct effect upon the excitability of the ventricle.

6. Attention is called to the importance of considering the relatively as well as the absolutely refractory phase in studies of functional cardiac changes.

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THE INFLUENCE OF HYPERPNEA AND OF VARIATIONS OF THE O_2- AND CO_2-TENSION IN THE INSPIRED AIR UPON AFTER-IMAGES

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In a preceding paper it has been shown that variations in the O_2- and CO_2-tension in the inhaled air brought about significant changes in hearing, which were attributed to alterations in the cortex of the brain. It seemed advisable to investigate whether similar changes could be obtained in experiments involving another sense organ and, therefore, a different cortical mechanism. For this reason the present study was undertaken in which the influence of CO_2 and O_2 was studied on a visual process. There are a few reports in the literature dealing with the effect of O_2-lack on vision. Most of them are incidental observations made during expeditions on high mountains (Hingston, 1925; Hartmann, 1933; and Barcroft, 1925). Quantitative studies by Wilmer and Berens (1918) showed distinct effects of O_2-lack on the visual field at an oxygen pressure corresponding to an altitude of 20,000 feet. Similar, but not very convincing results, were obtained by Goldmann and Schubert (1933). Bagby (1921) did not observe any changes in visual acuity, but Schubert in 1934 observed a considerable decrease in the distinction threshold for brightness.

Johnson and Paschal (cited by McFarland) believe that the effects do not concern the sensory mechanism, but are due to lack of "attention." There are no reports in the literature concerning the effect on vision of CO_2-excess or CO_2-lack, as is obtained in experiments with voluntary hyperpnea.

The influence of O_2-lack, CO_2-excess and hyperpnea was studied on after-images for two reasons: 1, they involve the fundamental visual mechanism; 2, they lend themselves to quantitative investigations (Gellhorn and Weidling, 1925; Gellhorn and Kühnlein, 1926) and appear, under suitable conditions, with a distinct and easily measurable latent period (Creed and Granit, 1928).

METHOD. The experiments to be reported in this paper were started after eight subjects had undergone a training period of several weeks, during which a very great consistency of the latent period of negative after-images was obtained when a colored square was fixated under stand-

ard conditions. The head was fixed in a head-holder, the eyes were closed for one minute prior to the fixation. The after-images were observed with closed eyes, and the latent periods measured with a stop-watch. The fixation was in most experiments binocular and in some monocular, without influencing the results. In the majority of the experiments a colored square (7.5 cm.^{2}) on a black and grey background was fixated at a distance of 60 cm. The illumination of the room was adjusted to the sensitivity of the experimental subject, so that latent periods were obtained which could easily be measured.

In a later series of experiments uncolored objects were chosen for after-image formation. A diaphragm of 10 cm. diameter forming the anterior wall of a wooden box which was illuminated from the inside was used as a pattern for after-images. The negative after-image consisted of a distinct black circle. O_2-lack and CO_2-excess were produced as reported in the first paper of this series. During the period of voluntary, maximal hyperpnea, which at the rate of 35 to 90 per minute lasted from two to six minutes, the respiratory minute volume was increased about 1200 per cent.

Results. A total of 62 experiments were performed, each of them consisting of a series of after-images obtained at intervals of at least 10 minutes, since it was found in preliminary experiments that in a trained subject the latent period remains very constant, provided that the interval is not shorter than 10 minutes. Several control experiments preceded and followed the observations during O_2-lack, CO_2- excess, etc.

The results of experiments on the effect of O_2-lack indicate that the effect varied with the O_2-concentration. Practically no change resulted from the administration of N_2-air-mixture of 13 to 16 per cent O_2. Occasionally a "supernormal" period was observed at the beginning of the O_2-lack period, which was apparent in a slight shortening of the duration of the negative after-image. In the major part of this series O_2 concentrations varying between 9.2 and 11 per cent O_2 were chosen (table 1). It was found that invariably under the influence of these greater degrees of O_2-lack the latent period of the negative after-image increased. Sometimes no after-image whatever was formed.

It is necessary to mention precisely the more outstanding subjective phenomena observed during these experiments. Ordinarily in most subjects upon closing the eyes a positive after-image was observed followed by a short dark interval. Hereafter a negative after-image appeared which was blurred at first, but developed very rapidly into one with sharp edges corresponding to the pattern of the object. It was this moment which was chosen for recording the latent period of the after-images. Under the condition of O_2-lack several changes in the appearance in the after-images were noticed. During the fixation of the colored square or of the white diaphragm the experimental subjects had the impression that the color

observed was less saturated and that the white diaphragm looked "as if the lights had been dimmed." After closing the eyes, the positive after-image did not appear at once, as it did in the control experiments, but there was a measurable latent period, and the positive after-image was considerably blurred. When, ordinarily after a dark interval, the negative

TABLE 1

The influence of O_2-lack, CO_2, and hyperpnea on the latent period of negative after-images

NUMBER	NAME	O_2-CONCENTRATION	DURATION OF O_2-LACK	LATENT PERIOD		
				Control before O_2-lack	At the end of O_2-lack period	Control after O_2-lack
		per cent	*minutes*	*seconds*	*seconds*	*seconds*
1	O.	10.2	8	15.3	∞	15.0
2	G.	9.8	7	3.1	∞	3.3
3	Sp.	10.5	14.5	5.1	22.4	6.2
4	St.	10.6	10	7.6	24.0*	6.0
5	G.	10.1	7	6.5	∞	5.9

NUMBER	NAME	CO_2-CONCENTRATION	DURATION OF CO_2-LACK	LATENT PERIOD		
				Control before CO_2	At the end of CO_2-period	Control after-CO_2
		per cent	*minutes*	*seconds*	*seconds*	*seconds*
1	Sp.	5.9	5.5	6.3	13.2	7.0
2	St.	6.4	5.0	11.5	∞	10.5
3	G.	6.0	5.0	2.3	3.7	2.7
4	F.	4.4	5.5	7.2	19.2	6.5
5	O.	4.8	4.5	17.0	∞	21.0

NUMBER	NAME	HYPERPNEA		LATENT PERIOD		
		Duration	Frequency per minute	Control before hyperpnea	At the end of hyperpnea	Control after hyperpnea
		minutes		*seconds*	*seconds*	*seconds*
1	Sp.	3	78	6.8	10.0*	7.7
2	G.	5	75	3.0	7.8*	2.7
3	St.	6	66	10.8	∞	9.9
4	O.	4	68	11.5	∞	15.0
5	F.	3.0	88	8.4	14.0*	10.8

* Experiment carried out several minutes after the end of O_2-lack period and hyperpnea respectively.

after-image appeared after a prolonged latent period, it was less saturated, of diminished intensity and duration. As mentioned above, it was found that under O_2-lack sometimes the after-images were so weak and blurred that they did not permit of a measurement of the latent period; moreover, not infrequently no negative after-images were formed.

As it is apparent from table 1, the effects were completely reversible, but it may be noted that a very considerable lengthening of the latent period was observed, even 10 or more minutes after the completion of the O_2-lack period (table 2).

Another series of experiments concerning the effect of CO_2-excess on after-images was performed. It was found that CO_2 in concentration below 4 per cent did not influence the appearance of after-images, although the respiration was greatly increased. If, however, concentrations above 4 per cent were administered, the after-images were altered in the same manner as was described following O_2-lack (table 1). The main difference between the two groups of experiments was a quantitative one, since the effects of O_2-lack were more pronounced than those of CO_2-excess. But even in this group distinct after-effects, that is, a lengthening of the

TABLE 2

TIME	LATENT PERIOD	TIME	LATENT PERIOD	TIME	LATENT PERIOD
minutes	*seconds*	*minutes*	*seconds*	*minutes*	*seconds*
1:55	13.5	11:08	11.0	10:13	6.2
2:06	13.0	11:18	11.7	10:27	6.4
2:18	13.0	11:28	12.0	10:44	6.6
2:23–2:49½	10.8 per cent O_2	11:33–11:37½	7.0 per cent CO_2	10:48–10:51	hyperpnea (rate 60 p.m.)
2:28	19.5	11:38	17.4	10:56	15.2
2:38	20.5	11:48	16.4	11:30	6.4
2:48	∞	11:58	12.4		
2:58	24.0	12:08	11.6		
3:08	12.4				
3:18	14.0				

latent periods, were occasionally observed after the end of the CO_2 period (table 2).

In a final series of experiments the influence of hyperpnea on after-images was studied. It was found that hyperpnea of one minute duration was ineffective in altering the after-images, while hyperpnea lasting from 2 to 5 minutes was regularly effective and led to a distinct increase in the latent period of negative after-images. The subjective phenomena (blurring; decrease in intensity) were similar to those described for O_2-lack. They occurred even at a time when the respiration was already restored to normal following a period of apnea (table 2).

The question arises as to whether the effects described are due to the influence of O_2 and CO_2 on the pupillary diameter, or concern the visual mechanism directly. For this reason experiments were performed in which only one eye was used and an artificial pupil was placed in front of the observing eye. The experiments indicate that even under these conditions

the same results are obtained. It may, therefore, be said that the influence of O_2-lack, CO_2-excess and hyperpnea on the after-images involve the visual mechanism itself.

Discussion. From the description of these results, it is evident that there is a great similarity between our previous experiments on audition and those on visual after-images. It was shown that O_2-lack, CO_2-excess, and hyperpnea led to a diminution of auditory acuity. The experiments with after-images indicate that in all three conditions the visual mechanism is likewise impaired. The appearance of the negative after-images corresponds to that obtained in experiments with a weaker stimulus, since obviously the duration and the saturation of the after-images was diminished. However, it is not improbable that the results indicate not only a very considerable decrease in excitability of the visual mechanism, but also a qualitative change, since in control experiments variations in the intensity of the stimulus never led to any considerable lengthening of the latent period. In fact, in several experiments the latent period was constant for various intensities until on further reduction of the stimulus it became infinite since no after-image was formed (in agreement with Juhasz, 1920).

In order to study the possible importance of vascular changes on the latent period and appearance of after-images, the blood pressure was measured in a number of experiments. It was found that O_2-lack did not alter the blood pressure in our experimental subjects, and the decrease of the blood pressure after hyperpnea amounted to not more than 6 mm. mercury and was very readily reversible. CO_2 led to increases of about 10 mm. mercury in systolic blood pressure. Control experiments in which, by means of physical exercise, the blood pressure was raised 20 mm. or more, show that blood pressure changes as such within the range observed in our experiments, have no influence on after-images.

Since O_2-lack and CO_2-excess cause vasodilatation, and since improved circulation would scarcely cause impaired function, the decrease in excitability which is apparent from the lengthening of the after-images must be due to chemical cellular changes, whereas the vasoconstriction accompanying hyperpnea and probably even outlasting it may fully explain the alterations in after-images described above. For a more complete discussion of the effect of hyperventilation the reader may consult the first paper of this series since the interpretation given there is equally applicable to the after-image experiments.

It should be mentioned that the results might be thought to be due to the inability of the experimental subjects to fixate properly. The long training makes this interpretation highly improbable. Moreover, control experiments in which the time of fixation was varied showed that with decreasing fixation time the latent period of negative after-images increases;

but it is very important to note that even the fixation during one second instead of 10, although it led to a lengthening of the latent period of the after-image, always caused a negative after-image, whereas, as reported in our experiments under the conditions of variations of the O_2- and CO_2-tension in the inspired air and after hyperpnea, the after-image was not infrequently absent. This indicates clearly that the effects of O_2-lack, etc., are due to an alteration in the nervous mechanism involved.

We, therefore, come to the conclusion that alterations in the CO_2- and O_2-tension of the blood, as are obtained by inhalation of various gas mixtures and by hyperpnea, lead to a decrease in sensitivity of the visual and of the auditory mechanism. The effects of these various factors are quantitatively but not qualitatively different. The only conspicuous difference between the auditory and visual experiments lies in the fact that we have not seen a shortening in the latent period of the after-images and an increase in their intensity following O_2-lack, CO_2-excess or hyperpnea. In other words, there is no equivalent for the temporarily increased auditory acuity. We are, however, not inclined to stress this difference because of the temporary nature of the increased excitability which may have escaped us for the reason that we could not perform the experiments more often than at intervals of 10 minutes. Moreover, it may be mentioned that, according to Schubert (1934), after a decrease in the distinction threshold for brightness during O_2-lack, a period of improved (above normal) brightness distinction was observed with readmission of air.

SUMMARY

The influence of O_2-lack, CO_2-excess and hyperpnea is studied in regard to the latent period and appearance of negative after-images It is found that under suitable conditions (O_2-concentration 9 to 11 per cent, CO_2-concentration 4 to 7 per cent, or voluntary hyperpnea lasting for 2 to 5 minutes at a rate of 35 and more per minute) there is a lengthening of the latent period of negative after-images or even complete absence of any after-images. The effect may last as long as 12 minutes after the readmission of air. The results correspond to those obtained in a previous paper with audition and seem to point out that O_2-lack, CO_2-excess and reduction in CO_2-tension of the blood due to hyperpnea each cause a lowered excitability of the fundamental nervous mechanism involved in vision.

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THE PHASIC CHANGES IN CORONARY FLOW ESTABLISHED BY DIFFERENTIAL PRESSURE CURVES[1]

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The question whether coronary flow under normal conditions is reduced or even stopped during systole, or, on the other hand, undergoes a marked acceleration, is still in dispute. Records obtained by flow-recorders of different design give quite different answers (Anrep and various associates, 1; Hochrein and associates, 2; Wiggers and Cotton, 3b; Gregg, 4). This divergence of opinion is occasioned by the fact that neither the theoretical principles according to which reliable instruments should be designed nor the tests by which their efficiency can be judged have been as clearly formulated as in the case of pressure recorders. Consequently their construction is guided too largely by empirical trials while judgment as to their efficiency remains essentially a matter of opinion.

Since consecutive changes in flow are the resultant of differences in pressure which exist from moment to moment in the peripheral and central ends of a coronary ramus and since the pressure variations in the central end closely follow those in the aorta (Wiggers and Cotton, 3a), the variations of flow through the intramural vessels of the heart should be determinable by the difference between aortic and peripheral coronary pressures recorded by calibrated optical manometers.

The limiting physical conditions for such deductions are *a*, that the peripheral pressure changes in coronary branches are essentially due to the effects of ventricular contraction and not significantly to communication of pressure from collateral vessels, and *b*, that peripheral coronary pressures (P.C.P.) can be recorded accurately *both* as regards contour and magnitude. In a previous paper (5) we presented evidence that transference of pressure from collateral channels is of no material importance in territory supplied by the ramus descendens anterior. Evidence was also presented that efficient optical manometers reproduce with accurate con-

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tour and time relations the peripheral pressure changes, but do not register the maximal systolic resistance which the myocardium is capable of exerting. Consequently it was necessary to devise expedients for determining the true maximum and minimum pressures with reasonable accuracy. Furthermore, since some investigators have questioned the validity of observations made upon hearts which are separated from their natural nervous control and perfused with liquids other than the unaltered blood of the animal, it is important that these determinations be made under conditions to which such criticisms do not apply.

PROCEDURE. The aortic pressure was recorded as previously by a calibrated optical manometer. The main branch of the ramus descendens anterior was exposed for a distance of about a centimeter by grasping the surrounding sheath with four mosquito forceps, incising it in a frontal line with a sharp knife and laying back the sheath. In this way the greater portion of the nerves accompanying the vessel are spared. An electromagnetic compressor was applied to the isolated ramus, so that the normal blood supply was maintained except for brief intervals of clamping described below. A second optical manometer was inserted into a side branch of the main coronary vessel just below the region of preparation.

The peripheral diastolic pressure and contour of the P.C.P. pulses were determined as in our previous publication (5), i.e., by recording pressures from a lateral branch of the ramus descendens anterior, while the main vessel was clamped for 4 or 5 beats. Unless the heart is very slow, stabilization does not occur for several beats after occlusion (fig. 1, A). Hence the diastolic pressure should not be read on the first beat. It is immaterial which subsequent one is selected as the diastolic pressures differ only by 2 or 3 mm. Hg. We have arbitrarily selected the pressure level just preceding the second of such beats.

In order to determine the true peripheral systolic pressure, lateral pressure curves were similarly recorded while the coronary artery was clamped centrally for brief intervals at a frequency slightly different from that of the heart beat. In this way fluid could be trapped in the coronary system at various pressures up to aortic systolic. The level at which such a closed coronary vessel just held pressure at a constant level during such clamping was taken as the critical value for maximum systolic peripheral pressure under the circulatory conditions existing at the time. While the procedure was similar to that used by Anrep and Saalfeld (1d) the data obtained served quite a different purpose.

The results of this procedure are illustrated in figure 1, B in which advancing periods of clamping (0.3 sec.) are marked in 5 beats. By considering these from right to left ($1 \rightarrow 5$) their significance is better understood. In beat *1*, clamping began late in diastole and extended well into systole. After an abrupt fall during diastole, the pressure rises during

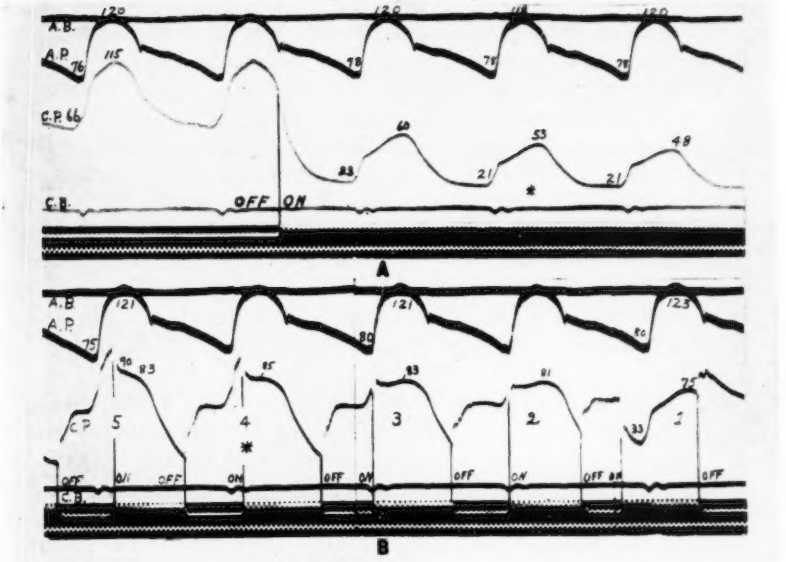

FIG. 1

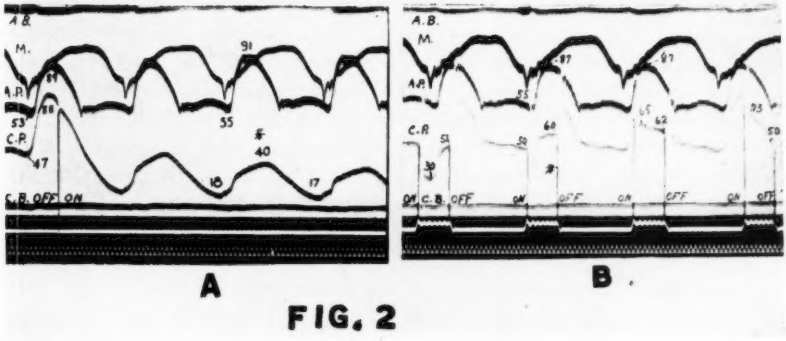

FIG. 2

Fig. 1. A, Record used for determining form of peripheral coronary pressure and the diastolic pressure. B, Record used for determining maximum systolic pressure by method of advancing short clampings. Description in text.

CP, coronary pressure; *AP*, aortic pressure; *CB*, coronary base line; *AB*, aortic base line. Periods of clamping in lower record denoted by "on" and "off." Time 0.02 second.

Fig. 2. Curves used for determining form and peripheral diastolic pressure (A) and systolic peripheral coronary pressures (B) under altered experimental conditions. Discussion in text. M, myogram; other lettering same as figure 1.

systole, reaching a height of 75 mm. The systolic part of the curve inscribed has the same form as that of a P.C.P. curve shown in figure 1, A. Although the beat develops from a relatively high diastolic pressure (33 mm. Hg) the systolic summit does not exceed 75 mm. Hg. In beats *2* and *3*, clamping occurred during the normal systolic rise of coronary pressure. In both instances a slight additional rise to 81 mm. and 83 mm. respectively occurs. In beat *4*, clamping took place after coronary pressure had exceeded such values. A pressure of 85 mm. is held during midsystole. In beat *5* the vessel was clamped nearer the systolic peak at 90 mm., but from this level it gradually declined to 83 mm. at the end of systole. Such a series of systolic clampings definitely shows that the maximum coronary resistance expressed in terms of peripheral coronary pressure is less than 90 mm. and greater than 83 mm.; in fact, it is about 85 mm. Hg.

It may be added parenthetically that a survey of all our records obtained under a great variety of conditions has never revealed an instance in which such maximum resistance ever approximated aortic systolic determined from simultaneous aortic pressure curves.

Critique of method. The question arises whether values for systolic P.C.P. so established truly represent the full magnitude of the pressure developed in the intramural vessels of the left ventricle. Various sources of error were considered and tested. The possibility that leaks in the manometer system might account for the drop of pressures when vessels were clamped at the systolic pressure peak was checked in each experiment by clamping the vessel both centrally and peripherally to the side branch and in some cases by introduction of dyes into the whole system. The area of distribution of the ramus descendens anterior precluded the existence of branches to the auricle such as Anrep and Saalfeld encountered in using the circumflex ramus. That a "runoff" occurred by arterial anastomoses with the left ventricle or other arterial branches (coronary or extracardiac) is improbable since the "holding" pressure was less than that in other arterial branches or the ventricle. Indeed, in a previous communication (5) we analyzed particularly the reverse possibility, viz., that pressure transmission may occur from arterial collaterals, but we could find no evidence of significant functional anastomoses in normal hearts. To check the possibility that tributaries of the ramus descendens anterior to the right ventricle may play a part in reducing the actual maximal pressure, a heart was selected in which several large branches of ramus descendens anterior appeared to be directed toward the right ventricle. Clamping of all these branches was, however, without effect upon the form of the P.C.P. curve or upon the maximal peripheral systolic pressure established by the method of advancing short clampings. Therefore no other conclusion could be drawn than that the pressure exerted upon intramural

vessels is definitely less than that created in the left ventricular cavity. Such relationships are physically understandable. The ventricular walls during contraction cannot be regarded as a semi-liquid substance in which pressures spread equally in all directions, but are more comparable to a laminated elastic ball under internal pressure in which the tensions are unequally distributed. While the theory of distribution of elastic stresses in such globular structures has not been reduced to mathematical terms, sufficient is known to square our observations with the physical possibilities. At the time of maximal contraction, the tension at any point within the myocardium can be resolved into three vectors. Two of these have a direction perpendicular to equatorial planes at right angles to each other and the direction of the third is parallel to the radius of the circumference.

The tension (F per unit area) developed perpendicular to either equatorial plane is that which tends to separate the two halves of the spherical shell containing fluid under pressure. It is presumably equal at every point and may be roughly calculated by the formula

$$F = \frac{PR^2}{R_1^2 - R^2}$$

in which P is the tension per unit area exerted upon the internal surface, R is the radius of the cavity of the sphere and R_1 is the radius to the outside wall. Assuming a reasonable capacity and thickness of ventricular wall during contraction it can be calculated that the tension per unit area is less in either equatorial plane than that exerted upon the internal surface, i.e., than intraventricular pressure.

The magnitude of the third vector (parallel to the radius) is not the same at all points. It is greatest in the internal layers and becomes progressively less toward the exterior where it is zero. In other words, a gradient occurs from the interior to the exterior, much as would be the case if the pressures could be measured between numerous layers of a large series of thin rubber balloons inflated from the interior and jointly bearing the pressure. Consequently the mean tension developed parallel to the radius is necessarily less than that exerted upon the inner surface, i.e., than intraventricular pressure. Actually the distribution of elastic stresses is probably far more complicated and we merely offer this physical analysis as a tentative explanation of our experimental facts.

Differential pressure plots. It should be apparent that two such records as are shown in figure 1, A and B, when taken in rapid succession supply all the data for construction of differential pressure curves. The reconstruction illustrated by curves of figure 3 was carried out as follows: The beat denoted by * in figure 1 A, and the corresponding aortic pressure curve were enlarged by optical projection upon cross-section millimeter paper

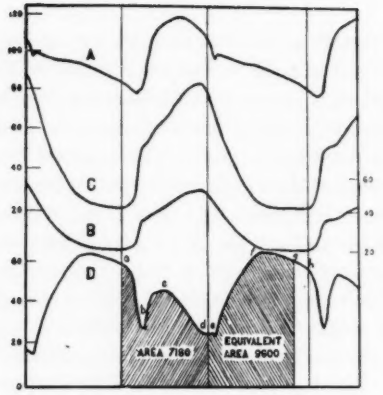

FIG.3

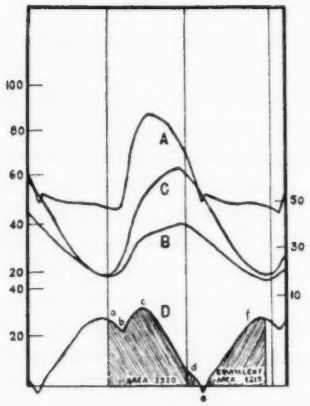

FIG.4

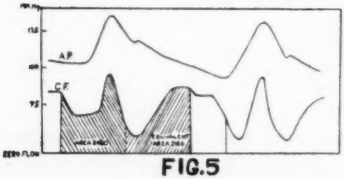

FIG.5

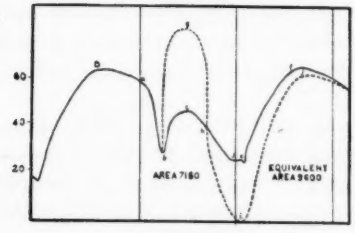

FIG.6

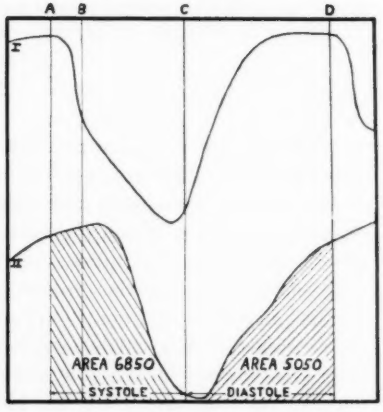

FIG.7

Fig. 3. Reconstructions from curves of figure 1 showing relative volume flows and velocity in intramural coronary vessels during systole and diastole. A, aortic pressure; B, peripheral coronary pressure; C, same, reconstructed with true ordinate values; D, velocity curve. Shaded areas, volume flow with inscribed numbers indicating planimeter measurements of areas on original enlarged graphs. Ordinates, millimeter Hg; abscissa, time. Discussion in text.

Fig. 4. Reconstruction graphs from curves of figure 2; lettering same as in figure 3. Discussion in text.

Fig. 5. Reconstruction of flow curves from Anrep, Davis and Volhard 1c, figure 8, arranged to read from left to right. Discussion in text. *AP*, aortic pressure; *CF*, coronary flow.

Fig. 6. Comparison of intramural coronary velocities (curve *D-a-c-e-f*) redrawn from figure 3 and possible inflow velocities into coronary artery (curve *b-g-i-j*). Discussion in text.

Fig. 7. Comparison of coronary intramural flow curves when artery is perfused under constant pressure. Curve I, our curve determined from curve C, figure 3; curve II, curve of Anrep, et al., 1a, fig. 4. Both curves redrawn to same coördinate scales. *A-B*, isometric contraction; *B-C*, ejection; *C-D*, diastole.

and redrawn. The P.C.P. curve recorded by a calibrated manometer, varying slightly in sensitivity from the aortic manometer, was first brought to the same scale as the aortic and then the true P.C.P. curve was constructed by increasing the ordinate values of the whole curve so as to bring the maximum pressure to the systolic level established in figure 1 B (85 mm. Hg). In actual practice the two steps were combined in one operation and are illustrated by curve C of figure 3.

The pressure differences between the aortic pressure curve (A) and the true peripheral resistance curve (C), when again plotted with respect to zero yield curve D. We consider this to represent the coronary velocity curve. It should be emphasized that it actually depicts the *velocity of the "runoff"* from the coronary arterial system and does not correspond in every detail with the *velocity of inflow* into the coronary orifice which is affected in addition by changes in capacity as coronary pressures increase or decrease. Since sources for the "runoff" other than intramural capillaries seemed to have been excluded by our previous studies, such curves represent the velocity of intramural flow. By drawing vertical lines such as *a*, *e*, *g*, to the zero base line and measuring with a planimeter the areas so bounded, values are obtained which, when compared, express reasonably well the *relative volumes* flowing during different periods of the heart cycle. Such comparisons are not quite exact owing to slight differences in the size of vessels in different phases of the cycle; but the error so incurred is within the range of accuracy in redrawing and plotting such curves.

The phasic variations of coronary flow under natural conditions. Curve D of figure 3 is submitted as exemplifying changes of flow through the ramus descendens anterior under natural conditions. The contour of the aortic pressure pulses and the ordinate values for diastolic and systolic pressure are within normal ranges. Unfortunately the heart rate in our experiments was generally higher than we wished—in this case 107 per minute.

An analysis of the velocity curve (D) shows that coronary flow is sharply reduced during isometric contraction (*a–b*), increases sharply during the early half of systolic ejection (*b–c*), reaches a maximum with the summit of aortic pressure (*c*) and then decreases considerably during the latter half of systolic ejection (*d*). During isometric relaxation the flow accelerates, reaching a maximum (*f*) in mid-diastole despite the gradual decline of aortic pressure. Following this the flow remains large but decreases gradually; in fact it essentially follows the diastolic pressure gradient. Grossly described, two distinct accelerations occur: a first (*c*) during systolic ejection, and a second (*f*) during diastole. The positive wave representing the first acceleration is situated in a systolic valley and the maximum velocity (*c*) is less than that at any diastolic point subsequent to isometric relaxation. Such relationships indicate that velocity of coronary flow is definitely decreased by contraction; but it is never zero.

Measurement by a planimeter of the areas under curve D representing systole (a–d) and diastole (e–h) yields an S/D ratio of $\frac{7180}{11460}$ or $\frac{1}{1.6}$. Similar comparison of the systolic area with an equivalent area of early diastole yields an S/D ratio of $\frac{7180}{9600}$ or $\frac{1}{1.34}$, thus indicating that the *volume flow* during systole is 75 per cent of that during an equal time interval of early diastole. It has generally been recognized that an increase in coronary peripheral resistance during systole accounts for the decreased systolic volume flow but a pressure curve such as is illustrated by curve C in figure 3 emphasizes the additional fact that the resistance decreases about as gradually during diastole as it increases during systole. In this way gradually diminishing resistance is offered to flow until resistance reaches a diastolic level. In other words, contraction operates to decrease flow during the early portion of diastole as well as during systole.

By methods just described it has been found that similar phasic variation and relative magnitudes of volume flow during systole and diastole also occur in the left circumflex ramus. Thus in one experiment the ratio of flow during systole and an equivalent portion of early diastole was $\frac{1}{1.35}$.

The phasic relations under altered dynamic conditions. Thus far we have emphasized that under essentially normal circulatory conditions the systolic volume flow approximates but never quite equals that in early diastole. In 1931 Hochrein (2) advanced the conception that the maximal flow may shift variously between systole and diastole under different circulatory conditions. While not subscribing to his experimental proof, our analyses indicate that the flow might be maximal in systole under circulatory conditions in which diastolic pressure is relatively low and pulse pressure large. Experimental proof that this can occur is offered in the curves reproduced in figure 2 and their reconstruction in figure 4. The differential pressure curve (D) obtained shows that the first systolic wave, c, reaches a higher peak than the subsequent diastolic maximum at f. Measurement of equivalent areas gives an S/D ratio of $\frac{2320}{1215}$ or $\frac{1}{0.52}$, i.e., the systolic volume flow is almost twice as large as the diastolic. Such experiments indicate that our current conceptions must be revised by admitting that dynamic conditions *can be created experimentally* in which the maximum volume flow shifts from diastole to systole.

Discussion. Our present analyses of flow through the intramural vessels under normal conditions depicted in figure 3 accord with observations of Wiggers and Cotton (3a) that lateral pressure curves give no evidence of decrease in coronary flow except during the period of isometric contraction when an extra preliminary oscillation occurs. The flow curves pre-

dicted by Wiggers and Cotton (3b) as a result of flow measurements under declining pressures also compare quite favorably. Even the sharp early systolic decrease in velocity missed by all previous observers was predicted. The investigators failed however to recognize the abruptness and extent of the systolic acceleration of flow and also the extent of its decrease during late systole.

Our interpretations of the systolic and diastolic coronary flow under natural conditions agree in part with those of Anrep and his associates; in several major particulars however our views are different. In our opinion these differences are partly attributable to use of different methods but many are merely a matter of interpretation. The cause of these differences, real and apparent, must be discussed, for no research is complete unless an effort is made to harmonize discrepancies. We are in fundamental agreement with Anrep and his colleagues 1, that ventricular contraction increases intramural resistance; 2, that *normally* the coronary volume flow is not greater during systole than diastole as claimed by German investigators, and 3, that, consequently, the beat of the ventricle mechanically reduces the minute volume flowing through the coronary vessels under otherwise identical conditions.

We feel however that the magnitude of the systolic reduction in flow was greatly over-estimated. In the first place distinctions between velocity and volume flow must be made. Our own curves show distinctly an initial sharp decrease in velocity followed by a subsequent increase which never attains the maximum found during late diastole. But despite these fluctuations, measurement of the areas beneath the curves shows that the volume flow during systole is still about 75 per cent of that during an equivalent interval of diastole.

If, as in figure 5, similar areas are measured under velocity curves published by Anrep, Davis and Volhard it is found that the *systolic inflow* into the coronary artery is even greater during systole than during an equivalent interval of diastole. Such curves are not quite comparable to our own, however. The systolic peak rises to a level higher than that of diastole owing to the fact that a component attributable to systolic expansion of the vessel is included. We cannot agree however that this systolic inflow volume is entirely determined by the elastic expansion of vessels. It is our impression that the clever experiments designed by these investigators to support such an argument may not have been as crucial as they believed. Thus they found that the systolic inflow is equal to normal even when coronary vessels are blocked by lycopodium. It must be recalled however that occluded vessels may accommodate more blood under the same systolic pressure in a main artery than when flow is unimpeded. This can easily be shown by placing a carotid artery in a plethysmograph and clamping the vessel peripherally.

An examination of the records published by Anrep, Davis and Volhard, one of which is redrawn in figure 5, gives, we believe, explicit evidence that a marked systolic intramural flow must have taken place. If the systolic part of flow curves were due wholly to capacity changes, then the curve should drop to a zero line at the point of maximum arterial expansion, i.e., at the peak of aortic pressure. *Since this is not the case,* but on the contrary the velocity never equals zero at any point, this must signify that some fluid was also moved through the intramural vessels during systole.

We have attempted to show by the dotted part of the curve in figure 6 the maximum extent to which our own curves of velocity might have been modified if the elastic accommodative changes during ejection had been added. Such superposition depicts at a glance how inflow velocities and outflow velocities of a coronary vessel may differ in phase without affecting the total volume flow per beat. This superimposed curve was obtained by assuming a flow of 0.3 cc. per beat and calculating the additional "probable increase" in capacity (determined from previously published volume-elasticity curves) at the peak of aortic pressure, then spreading the curves so that the area gained under *b–g–h* is subtracted by *h–i–j*. Such curves resemble the coronary inflow curves of Hochrein and associates even more than those of Anrep, Davis and Volhard. They emphasize how misleading interpretations of intramural flow based upon changes in velocity at a coronary orifice may become.

The flow at constant perfusion pressures. Since for technical reasons most of the studies on coronary flow require perfusion of the vessels with blood or oxygenated saline solution under a *constant* pressure and since Anrep and his associates are inclined to regard flow curves obtained under these conditions as most trustworthy it seems advisable to compare their curves with those predictable from curves of P.C.P. redrawn to a true ordinate scale. If in relation to curve C, figure 3, we assume a constant perfusion pressure above the maximum P.C.P. the flow curve should be represented by an inversion of this curve. Such a curve, together with a copy of the flow curve of Anrep et al. (1a) are redrawn in figure 7. The essential differences are 1, an earlier retardation of flow during systole, and 2, an earlier acceleration and a quicker return to a maximum in diastole in our curves. In brief, if the curve II were advanced 0.02 to 0.04 second it would show a general correspondence to our own. These differences, though apparently minor, are nevertheless important.

If we were likewise to determine the flow curves at progressively lower perfusion pressures from curve C, figure 3, it is obvious that the initial retardation would *always begin* with the isometric contraction and not progressively earlier as claimed by Anrep and Häusler (1b). Since we have shown that the early retardation of flow is due to a rise of myocardial

tension during isometric contraction it is difficult to conceive of any factual reason why the time of such retardation should be a function of the perfusion pressure. Anrep and Häusler's results are more probably due to progressing hypodynamic states associated with slower development of intraventricular and myocardial tensions combined with a natural lag of the flow-recording apparatus as a whole.

As regards the magnitude of the decrease in flow during systole when the vessels are perfused at constant pressures both curves of figure 7 show that the velocity of flow diminishes rapidly. According to our evidence systolic flow is never arrested, even momentarily, unless perfusion pressures are used which are below the maximal peripheral coronary pressures (e.g., 85 mm. Hg in the record under discussion). According to the curves of Anrep and his various associates complete arrest can occur momentarily somewhere toward the end of systole, even when perfusion pressures 50 mm. above aortic-systolic are employed. The fact that at most a momentary arrest occurs and that a considerable volume flow is nevertheless maintained is obvious when the areas determined by such curves are measured. As shown in figure 7, the systolic volume flow is even greater than during an equivalent period of early diastole (ratio 1.36:1.0). This fact which was most certainly evident to Anrep and his various associates was never strongly emphasized; on the contrary, readers of his articles who have no clear appreciation of the graphic evidence gain the impression that complete cessation of flow occurs for all or a large part of systole. Thus in their last article (1d) the phraseology again appears, "the blood supply to the heart *during systole* is negligible." It is regrettable that such unfortunate phraseologies have been repeatedly copied and transcribed by writers when in fact the actual records show that the volume flow during systole is as large as during early diastole when the vessels are perfused under constant pressures.

SUMMARY

Under experimental conditions which permit retention of a natural blood supply under pulsatile pressure and which spare the innervation of blood vessels, we recorded the form and magnitude of the central coronary pressure (aortic pressure) and by special expedients, detailed in the text, also the contour and magnitude of the peripheral coronary pressure. The differential pressure curve constructed from these quantitated records gives a *velocity* curve of intramural flow and the areas beneath different portions of such a curve with zero pressure as a base line allow comparison of the relative *volume flows* during various portions of the heart cycle.

Such curves show that under normal conditions the velocity of intramural flow is abruptly decreased during isometric contraction; the flow

accelerates while aortic pressure rises to its summit, again to be retarded during the last portion of systole. A great acceleration occurs during isometric relaxation and a maximum velocity is attained at about the time the ventricles begin to fill during diastole. During the remainder of diastole, the velocity gradually decreases, the degree of retardation at the end of the cycle depending upon duration of diastole and the gradient of the drop in aortic pressure.

The changing resistance within the ventricular walls and the variations of aortic pressure during each cardiac cycle constitute continually opposing forces which determine the velocity of flow from moment to moment; but one or the other dominates at different times during each heart beat. The rapidly increasing resistance previous to the systolic elevation of aortic pressure accounts for the brusque retardation of early systole. Similarly the rapid decrease in myocardial resistance accounts for the prompt acceleration of diastole flow before ventricular filling begins. The secondary acceleration during systole as well as the continued high velocity during most of diastole are dominated by the aortic pressure.

A study of our curves shows that these fluctuations in *velocity of flow* during the cardiac cycle give no information regarding the *volume flow* during systole and equivalent periods of diastole. *Actual comparisons of such volume flows show that the myocardium receives approximately three-fourths as much blood during systole as during an equivalent interval of early diastole, but of course much less than during the entire period of diastole on account of its greater length.* Under exceptional experimental conditions characterized by relatively low diastolic pressures and large pulse pressures the systolic volume flow may even exceed that of equivalent periods in diastole.

Neither the phasic velocity nor volume flow *from* the larger coronary rami into and through the capillaries of the myocardium correspond exactly with the velocity or volume flow *into* the mouth of a coronary artery, for as Anrep, Davis and Volhard correctly emphasized the rate and volume of inflow are modified by changes in the capacity of the coronary vessels as the arterial pressure increases or decreases. It is therefore impossible as Hochrein and Keller have done to predict systolic and diastolic variations in intramural volume flow from velocity curves obtained at the mouth of an artery. The curves of figure 6 show how greatly the curves may differ. On the other hand, we have presented evidence of our own and point out suggestive evidence in curves published by Anrep, Davis and Volhard which indicates that the increased volume entering the coronary system during systole is not retained until it is moved onward during subsequent diastole; on the contrary a part of this systolic increase is moved forward through intramural capillaries during the period of systolic ejection.

An analysis of the changes in peripheral coronary resistance indicates that, contrary to general belief, the tension exerted by the myocardial contraction upon the intramural vessels is never as great as aortic systolic pressure. Consequently it is our belief that when coronary vessels are perfused under constant pressures, the flow is also not arrested—even momentarily—unless perfusion pressures very definitely below aortic-systolic are employed. Even in such curves, as in records presented by Anrep and his associates, the evidence is clear-cut that the *volume flow* during systole is quite as large as during an equivalent period of early diastole. *Physiologically it is more important to stress the maintenance of a good systolic volume-flow than the decreasing velocity and possible momentary arrest toward the end of systole, when vessels are perfused at constant pressures.*

Any interpretation or statement regarding coronary flow which implies that the myocardium receives only a negligible supply of blood during contraction —regardless of whether constant or pulsatile pressures are operating—is contrary to all hemodynamic evidence, that submitted by others as well as ourselves.

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THE PERIPHERAL MOTOR SYMPATHETIC INNERVATION TO AND WITHIN THE UTERUS[1]

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Several recent reviews attest to the fact that the peripheral innervation to the uterus and the nature of its intrinsic innervation are not understood (Davis, 1933; Gruber, 1934; Fontaine and Hermann, 1932). Indeed, Davis, who has summarized most of the contributions reported in the literature, concludes that "research by a considerable number of the most eminent authorities has failed to establish some of the most important details concerning the exact anatomy of the intrinsic nerve-supply of the uterus." It is generally agreed, however, that the uterus has within its walls an abundance of nervous tissue, richer about the utero-vaginal junction than elsewhere. Except for this region, the uterus of the rabbit is virtually devoid of intrinsic ganglia (Dr. A. A. Davis and Prof. W. P. Kennedy, personal communication). The morphological features of this intrinsic uterine innervation are totally unknown. Langley and Anderson (1895–96) showed many years ago that the motor sympathetic innervation to the uterus is through the vesico-uterine strands of the pelvic plexus, but the nerve pathways to the uterine cornua from this point have not been determined.

In the course of certain work relating especially to the hormonal control of uterine motility in transplanted uterine fistulae, it became desirable for us to know certain facts concerning the distribution of these nerve paths from the pelvic plexus to their respective, ultimate destinations. Accordingly, the following experiments have been performed. The experimental procedures adopted were based upon the possibility that in the rabbit nerves might pass from the vesico-uterine strands through the parametrium and send off fibers more or less locally to the uterus along the whole course of the parametrium.

PROCEDURES. The results reported below are based upon observations made on eleven mature, ovariectomized rabbits. In six of these, both uteri were used. Thus

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the experimental procedures were carried out on seventeen uteri. The innervation to the cervical region was examined in eight of these. At every step in the following procedures uterine responses to presacral nerve stimulation were obtained repeatedly, two, three, or even more times.

The rabbits were anesthetized lightly with Dial (Ciba), 0.6 cc. per kgm. being given intraperitoneally. The uterus was exposed through a low midline incision and

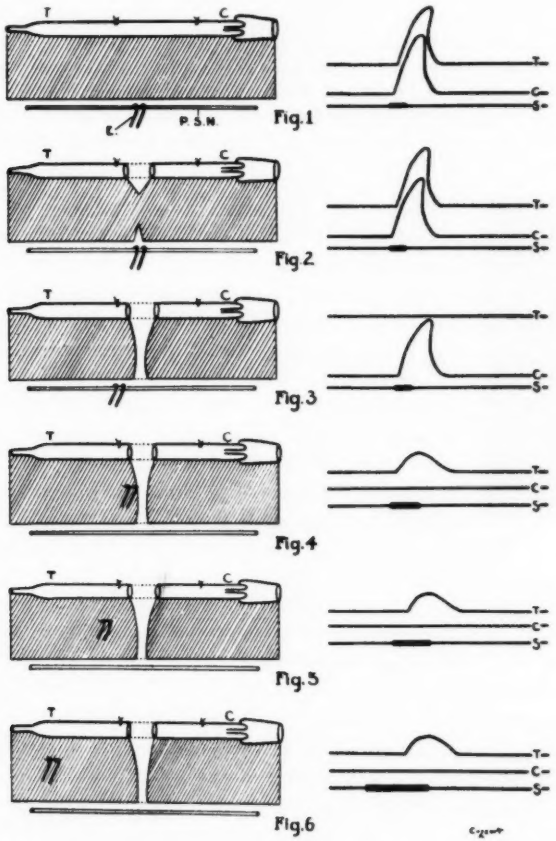


Plate I. Schematic representation of experimental steps performed (on left) and of results obtained (on right) in showing *the non-essentiality of uterine continuity for responses of the whole organ to pre-sacral nerve stimulation.* See text for discussion. Legend for this and other figures:

T, tubal end of uterine cornu to which thread for recording myographic activity is attached. *C*, cervical end of uterine cornu, to which a second thread is attached. *E*, stimulating electrodes. *P. S. N.*, pre-sacral nerve. *S*, stimulation signal. ↑, epinephrine administered intravenously.

two threads, each connected with a long, light lever, were passed through the uterus. One of the threads was attached near the point of fusion of the two uterine cornua (*C*, plate I, no. 1), and the other, somewhat above the mid-portion of the uterus toward the tubal end (*T*, plate I, no. 1). The several strands of the pre-sacral nerve were carefully exposed about the level of L_5 and placed in a shielded electrode. Faradic stimulation of moderate strength was employed. Records of contractions from the two portions of the uterus were recorded in the manner shown in text figure 1. Time records were dispensed with since only the qualitative results were desired. In every case, however, the period of stimulation lasted for approximately 15 seconds.

After three to six initial uterine responses to presacral nerve excitation had been elicited, the parametrium and uterus were exposed in the mid-region between the points at which the threads were attached. The vascular pattern was studied and if, as sometimes happens, there was but one large vein draining the terminal end of the uterus and it lay across this region, it was spared as the parametrium was divided in the manner described below.

Text fig. 1. Excerpts from experiment on which diagrammatic results of plate I are based.

RESULTS. *Non-essentiality of uterine continuity for responses of the whole organ to pre-sacral nerve stimulation.* (Plate I; text fig. 1.) In this group of experiments the uterus was first semi-sected in the mid-region and cut completely through in the second step (plate I, no. 2). Following this, the parametrium was severed progressively in small steps of 5 to 10 mm. each. All such transections of the parametrium were made between two ligatures passed in appropriate steps at the level at which the tissue was to be divided. Between any two such successive steps the pre-sacral nerve was stimulated and uterine responses repeatedly obtained or attempted. As soon as a cut in the parametrium resulted in abolition of responses in the terminal, or tubal end of the uterus following stimulation of the pre-sacral nerve, electrodes were placed at this most recently cut portion of the upper parametrium and responses of the terminal end of the uterus obtained (plate I, no. 4).

In this series of experiments, it was found that the uterus and approximately one-half to one centimeter of the adjacent parametrium could be transected *without* affecting responses of the upper segment of the uterus when pre-sacral nerve was stimulated (plate I, normal, no 1; uterus severed, no. 2: text fig. 1, normal no. 1; uterus severed, no. 2). It was not until the parametrium had been cut through, usually in the second centimeter from the uterus (or approximately in the middle third of the parametrium) that a dissociation of responses occurred between the two segments of the uterine cornu when the responses were abolished in the upper but persisted in the lower segment (plate I, no. 3; text fig. 1, no. 3).

Demonstration that principal nerve paths lie wholly within the parametrium. (Plate II.) This point was investigated in the following manner. After

normal uterine responses to presacral nerve stimulation (plate II, no. 1) were obtained, the parametrium was divided progressively in small steps from the uterus to the retroperitoneal wall, or the transection was commenced at the retroperitoneal wall and continued toward the uterus. In still other experiments the transection was commenced in the middle of the parametrium, but *in every case the uterus*, with a small bit of adjacent parametrium, *was kept intact* throughout an experiment.

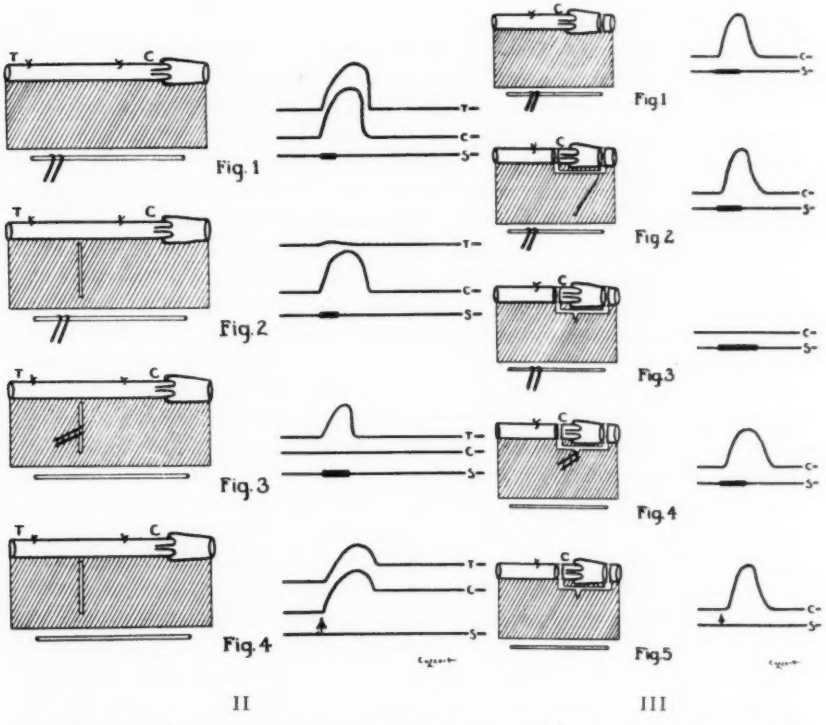


Plate II. Schematic representation of steps performed showing that *the main motor nerve paths to the whole uterus lie in the parametrium.* See text for description.

Plate III. Schematic representation of steps showing *the non-essentiality of the utero-vaginal junction* (with *ganglia uterini cervicalia*) *for nerve responses of the adjacent uterine cornua.*

In all of these experiments, a point on the parametrium was eventually reached, transection of which abolished responses of the terminal or tubal end of the uterus lying above the level of the cut (plate II, no. 2). This

point, as mentioned above, lies approximately midway between the retroperitoneal wall and the uterus. When electrodes were placed at the distal cut edge of the parametrium at the site where the last transection abolished the responses of the terminal end of the uterus to pre-sacral nerve stimulation, a contraction of the corresponding end of the uterus was obtained (plate II, no. 3). In order to show in certain experiments that in spite of some slight congestion the circulation was still good, epinephrine was administered intravenously (plate II, no. 4). If, as the result of epinephrine injection, the tubal end of the uterus failed to contract, the previous records were discarded.

These experiments show, therefore, that uterine continuity is not essential for pre-sacral nerve responses of the uterus but the integrity of part of the parametrium is essential. Thus the main nerve paths connecting the upper and lower portions of the uterus lie wholly within the parametrium.

The local distribution of nerves to the uterus from the parametrium and the extent of the intramural plexus. This point was approached by several aspects of our experiments and in such a way that certain conclusive statements may be made regarding it. In the first place, it was pointed out above that transection of the parametrium alone, leaving the uterus itself uncut, effected in the uterus above the level of section a loss of responsiveness to pre-sacral nerve stimulation. Thus we observe that one portion of the uterus is quiescent while another part, in structural continuity with it, contracts. Since Langley and Anderson showed that the sympathetic nerves to the uterus of the rabbit contain no inhibitory fibers,[3] and since in these experiments uterine continuity is unimpaired, our results show that there is no intra-uterine innervation (plexus) capable of bridging the gap made by cutting the parametrium alone. It must be mentioned, however, that a delayed response in the upper portion of the uterus may occur if the ovariectomized rabbit has been treated with oestrin, in which case the uterus is very irritable. Such delayed contractions are due to propagation of visible, slow waves of muscular contraction, *and in this respect differ from the simultaneous contraction of the whole organ* that one observes if the pre-sacral nerve is stimulated when the parametrium is intact.

Other of our data are even more convincing, however, in showing that the local nerve supply to the uterus is by collateral branches from the main parametrial nerves. In certain experiments (in which the rabbits

[3] Actually the responses one obtains to hypogastric nerve stimulation will depend upon the species and sexual state at the time of stimulation (Sauer, Jett-Jackson, Reynolds, 1935). The argument is not changed, however, since the uterine fibers are not mixed, i.e., excitatory *and* inhibitory.

have not been ovariectomized too long) one may obtain responses of the uterus by stimulating the parametrium directly. When this is done, the records one obtains are usually slow to reach a peak and to relax. The duration of the latent period in such experiments is determined by the relation of the point of stimulation to the point (thread attachment) from which the myogram is obtained. This is exemplified in the figures shown (plate I, nos. 4, 5, 6: text fig. 1, nos. 4, 5, 6). Here it may be seen that the latent period of no. 5 is double that of no. 4, and that of no. 6 is over four times as long as no. 4. The position of the electrodes in each case is indicated in the corresponding figures of plate I. In experiments such as these, it was observed that the uterine contractions commenced approximately opposite the parametrial site of stimulation, and from this position the wave of blanching and constriction spread slowly in both directions. It thus appears that the effect of parametrial stimulation is to initiate a local contraction which spreads by muscular conduction. This effect is seen to the best advantage when the stimulation is applied at the uppermost end of the parametrium, and differs markedly from the nearly simultaneous contraction of the whole uterus which results from stimulation of the pre-sacral or a main parametrial nerve. *The inference is clear*, therefore, on the basis of these observations, *that the motor sympathetic nerve fibers that enter the uterus from the parametrium do not innervate distant parts of the organ, but supply it only locally.*

In further support of this conclusion it should be mentioned that in those rabbits which have been castrated for some time, the local uterine contraction resulting from stimulation of the most distal end of the parametrium may be definitely seen, but the contraction so initiated dies out before reaching the site from which the records are obtained. Thus failure to record a contraction with this method of study is not to be construed as a negative result unless the whole uterus has been observed. Moreover, animals with oestrin, whether from their own ovaries or as a result of injection, are not suitable for these studies since the uterus is so irritable that spontaneous movements interfere with the observations. Still another factor to be taken into consideration is that the effects described above are to be seen advantageously only in rabbits whose uteri are long. With short cornua, distinctions in latent period of response to parametrial stimulation at selected sites are difficult to discern.

The non-essentiality of the utero-vaginal region for nerve responses of the adjacent uterus. (Plate III.) The relationship of the richly innervated utero-vaginal junction to the immediately adjacent uterine cornua was investigated. This was done to ascertain the essentiality or non-essentiality of this tissue for the uterine responses to pre-sacral nerve stimulation. Records of the lower uterine segment (C) were obtained under the three

following conditions: first, when the vagina was transected (plate III, no. 1); next, when the utero-vaginal junction was completely and carefully excised (plate III, no. 2), and finally, when a small incision was made in the parametrium at the level of the cervix (plate III, no. 3).

The results of these procedures showed that transection of the vagina does not abolish responses of the uterine segment just above the utero-vaginal junction, nor does careful excision of this whole region affect the nerve responses of the cornua (plate III, nos. 1 and 2). *Thus these data show that the principal nerves to the uterus do not go by way of the ganglia uterini cervicalia, since the tissue containing these structures may be removed without abolishing pre-sacral nerve responses in the uterine cornua.*

If one now makes a small cut approximately 2 to 3 mm. long at the margin where the utero-vaginal tissue was excised and at the level of the cervix prior to its removal, it is found that pre-sacral nerve stimulation now fails to elicit a response (plate III, no. 3). Stimulation of the peripheral cut margin at this spot will, however, elicit responses (plate III, no. 4). This point, where a small cut severs the main nerves to the uterus, is the approximate place indicated by Langley and Anderson at which they terminated their study of uterine innervation (Langley and Anderson, 1896, their diagram on p. 389). If one holds this portion of the parametrium to the light, he may see grossly these principal nerves which come from the pelvic plexus. The present observations testify, then, to the non-essentiality of the uterine cervical region and the adjacent vagina for uterine responses to pre-sacral nerve stimulation, but the main uterine nerves do pass very close to it.

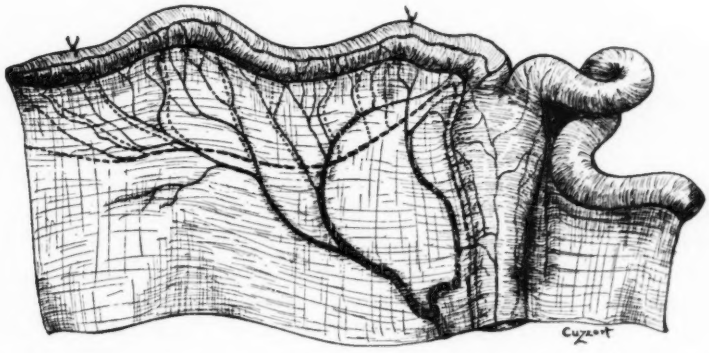
(It should be mentioned that the amplitude of the recorded contractions may diminish considerably upon transection of the vagina. If one sews the cut edges together the pull of the lower vagina is again exerted, but the nervous connection between the two portions has been severed. Such precautions must be exercised throughout work of this sort, else one may read into the results physiological inferences which are not warranted.)

CONCLUSION

The foregoing account forces us to adopt a definite picture of the peripheral motor sympathetic innervation to and within the uterus of the rabbit. This we have represented in text figure 2. The vesico-uterine strands from the pelvic plexus give rise to nerve fibers that pass close to the utero-vaginal junction and then dip more or less deeply into the parametrium as they run cephalad. Along their whole course they give off fibers which pass to the uterus. These collateral fibers innervate rather restricted regions of the uterus and do not contribute to a widespread, diffuse plexus within the uterine walls. This also applies to the highly nervous utero-

vaginal and uterine cervical portion of the genital tract, despite the presence of the uterine cervical ganglia.[4]

Finally, it should be mentioned that we have confirmed the observations of Langley and Anderson, that in addition to the motor fibers, vaso-constrictor fibers in the uterus are also stimulated upon excitation of the pre-sacral nerve. We have also observed in chronic experiments on cervicectomized rabbits that pallor (a mottling) of the uterus may occur when the pre-sacral nerve is stimulated, without myometrial contraction after



Text fig. 2. A uterine cornu showing the parametrium, the larger blood vessels and, by dotted lines, the general position in which the main motor sympathetic nerves to the uterus are found. When the uterus is short the nerves lie somewhat closer to the uterus than indicated here. See text for discussion of the motor nerves, the vasomotor nerves and of the intrinsic innervation of the uterus as well.

the main motor fibers to the uterus have been severed. Thus the vasomotor innervation to the rabbit uterus reaches this organ by paths other than in association with the peripheral motor innervation. Admittedly without proof, we tentatively suggest that the vaso-constrictor fibers to the uterus pass to their ultimate destination in close association with the vessels they innervate and which in this work remain intact.

[4] It should be noted in passing that Langley and Anderson found that stimulation of the dorsal-most vesico-uterine strand causes contraction of the terminal half to two-thirds of the uterus; stimulation of the middle vesico-uterine strand causes contraction of the lower third to half of the uterus, and excitation of the ventral-most strand causes contraction of the lower vagina. We have made no attempt to confirm this observation, and for that reason have chosen to represent in text figure 2 the parametrial nerves as a dotted line to indicate the principal course of the many nerves in this region, rather than their number or actual distribution.

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THE INFLUENCE OF PROGRESSIVE TOXEMIC LIVER DAMAGE UPON THE DEXTROSE TOLERANCE CURVE[1]

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Previous work has shown that the pancreas is not essential to the metabolic reactions determining the normal dextrose tolerance curve, while the presence of the normal liver is essential (1) (2). Our results indicated that the normal liver responds to an increased blood sugar, resulting from sugar administration, by decreasing its output of sugar which it was previously supplying to the blood from its own resources. That is to say, the administered sugar acts as an inhibitory stimulus to the liver so that the exogenous sugar supply temporarily replaces the normal endogenous supply, thus leading to the characteristically rapid disposal of the extra sugar and the return of the blood sugar to the normal level. This homeostatic liver mechanism has recently been confirmed by Tsai and Yi (3) who quantitated the sugar entering and leaving the liver by means of a flowmeter.

It has also been demonstrated that toxemia, which renders the dextrose tolerance curve abnormal in the intact animal, produces the same results in the complete absence of the pancreas under our experimental conditions (4). It was concluded that the abnormal dextrose tolerance curves occurring in toxemia are due to the effects of the toxin upon the liver and not the pancreas, and that the toxin interferes with the homeostatic liver mechanism which we have described.

The abnormal dextrose tolerance curves obtained in our previous toxin studies were all "diabetic" in character. We used large intravenous doses of diphtheria toxin in order to produce rapid and pronounced toxic effects. It has been shown, however, by Althausen and others (5), that in less acute toxemias where there is a longer survival period, the "diabetic" type of curve may give way to the "supernormal" before death intervenes. Clinically this variation in the abnormal curve caused by liver damage has been described by Judd, Kepler and Rynearson (6), and it is well known that "diabetic," "supernormal" and even normal dextrose tolerance curves

[1] Presented before the American Physiological Society, April, 1935.

[2] Supported by the Max Pam Fund for Metabolic Research.

may be obtained in cases of liver injury, without apparent relation to the degree of liver damage as judged by clinical or pathologic criteria. Indeed, this lack of correlation has been reported by Mann as applying to other tests of liver function (7).

In view of our previous work, we therefore thought it desirable to perform repeated dextrose tolerance tests throughout a progressive liver damage caused by toxemia, in an attempt to correlate the variations of the abnormal curve with the homeostatic liver mechanism described above.

METHODS. All these experiments were performed on unanesthetized normal dogs which were trained to lie quietly on an animal board while the injections and blood sampling were done by means of venous puncture with hypodermic needles. Food was withheld for 18 hours prior to each experiment and the test sugar was always given intravenously.

As in the previous work, toxemia was produced by the intravenous injection of diphtheria toxin.[3] Since we desired a more slowly progressive toxemia than in the previous experiments, we employed a dose of 4.8 minimum lethal doses per kilogram body weight, which had been found by preliminary experiments to result in death of the animal in about 10 hours.

After a preliminary control dextrose tolerance test, the toxin was administered and the tolerance curves repeated consecutively until exitus intervened. Three series of tests, using different amounts of test sugar, were performed. In the first series, 1.75 grams sugar per kilogram body weight was administered at intervals of 2 hours. In the second and third series, 0.9 and 0.25 gram sugar per kilogram body weight respectively were given at hourly intervals.

The course of repeated tolerance tests, with the above doses of sugar but without toxin administration, and the effects of the injection of the experimental dose of toxin without the tolerance tests, were observed in a number of control experiments.

RESULTS. The nature of these experiments necessitated the determination of so many blood sugar values that it is impractical to present our data in tabular form. With minor variations, the results of each type of experiment on a number of animals agreed within narrow limits. Typical results are graphically illustrated in figures 1 to 3, and are grouped so as to facilitate comparison.

Dextrose tolerance curves obtained with 1.75 grams dextrose per kilogram body weight. Figure 1 presents typical successive dextrose tolerance tests, with (—●—) and without (- - ● - -) the administration of toxin, when the test dose of dextrose was 1.75 grams per kgm. body weight. The effect of the same dose of toxin without sugar administration (—○—)

[3] We are indebted to the Eli Lilly Co. for a supply of standardized potent diphtheria toxin.

is also included. Toxin alone is followed by a small but sustained rise in the blood sugar level lasting for about 6 hours, at the end of which time the level begins to fall very slowly for another 2 hours. In the final 2 hours there is a precipitous fall of the blood sugar to hypoglycemic levels, accompanied by typical hypoglycemic convulsions. The nature of this final phase is such that it can only be ascribed to a sudden and complete failure of the glycogenic functions of the liver, the organ which has been shown to be the only endogenous source of blood sugar (8).

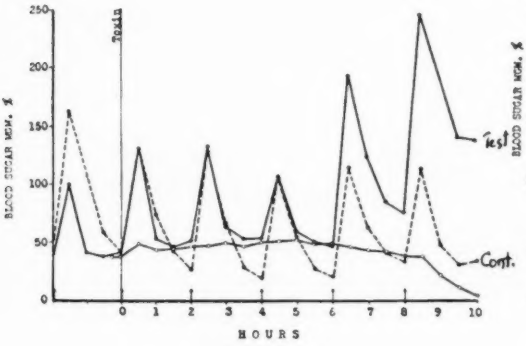

Fig. 1

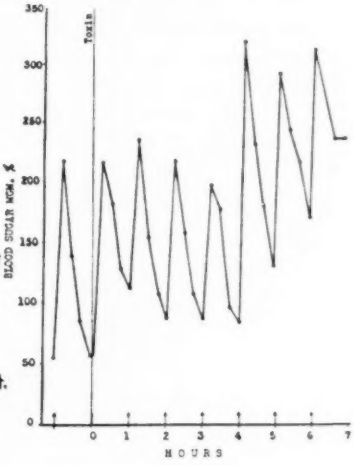

Fig. 2

Fig. 1. Successive dextrose tolerance curves, obtained with 1.75 grams dextrose per kgm. body weight, administered intravenously. Arrows represent sugar administration.

- - ●- - - - ●- - Control series; no toxin given.
—●——●— Toxin given after single control curve.
—○——○— Effect of toxin alone; no sugar given.

Fig. 2. Successive dextrose tolerance curves, obtained with 0.9 gram dextrose per kilogram body weight, administered intravenously. The initial control curve is followed by toxin administration. Arrows represent sugar administration.

The control series of dextrose tolerance curves, when no toxin is given, shows progressively lower peaks and greater overswing below the initial blood sugar level, until the fourth curve is completed. The subsequent curves show a cessation of the increase in tolerance, although both curves remain within normal limits. We have evidence to show that such curves may be repeated for 24 hours and still remain within these same limits.

The dextrose tolerance curves obtained after toxin administration do not show the normal increase in tolerance, although they remain within

normal limits. The last two curves, however, which parallel in time the falling blood sugar level caused by toxin alone, show a sudden change to the "diabetic" type of response which continues until exitus supervenes. The failure of the earlier curves in this series to show the normal increase in tolerance might be interpreted as a slight "diabetic" tendency, but might also be ascribed to a masking effect of the rise in the basic blood sugar level caused by the toxin itself. The latter relationship, however, can not be postulated for the final two curves since they proceed in a direction opposite to the toxin curve. It seems clear that these final "diabetic" curves are related to the failing hepatic function in accord with our previous observations on the "diabetic" type of dextrose tolerance curve obtained with the same test dose of sugar in hepatectomized animals.

Dextrose tolerance curves obtained with 0.9 gram dextrose per kilogram body weight. Figure 2 presents a typical series of dextrose tolerance curves

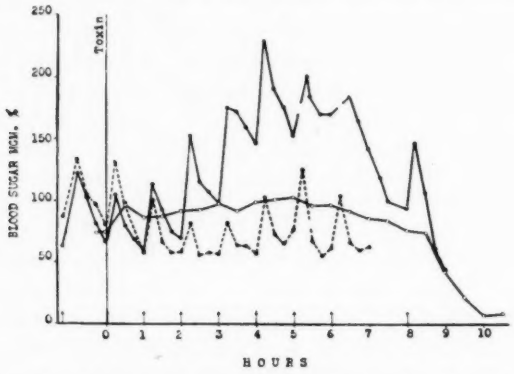

Fig. 3. Successive dextrose tolerance curves, obtained with 0.25 gram dextrose per kilogram body weight, administered intravenously. Conventions as in figure 1.

obtained after toxin administration, when the test dose of sugar was 0.9 gram per kgm. body weight. As the control test before toxin administration shows, it was possible to administer this smaller amount of sugar every hour and so obtain more frequent curves. It should be noted that the administration of the toxin causes an immediate "diabetic" type of change in the character of the subsequent tolerance curve, but that the repetition of the sugar dosage returns and holds the curves at a normal contour until the final "diabetic" sequence, coincident with liver failure, occurs.

Dextrose tolerance curves obtained with 0.25 gram dextrose per kilogram body weight. Figure 3 presents typical successive dextrose tolerance tests, with (—●—) and without (- - ● - -) the administration of toxin, when the test dose of sugar was 0.25 gram per kgm. body weight. Again, as in figure 1, the effect of toxin alone (—○—) is included for comparison.

With this small dose of sugar, the "diabetic" nature of the second curve, after toxin is very apparent, and this type of curve persists until the fifth curve is reached when (as occurred much earlier with 0.9 gm. dose) the normal response reappears. However, here the resemblance to the previous series ends, for, instead of "diabetic" tolerance curves, "supernormal" curves are obtained during the period coinciding with hepatic failure.

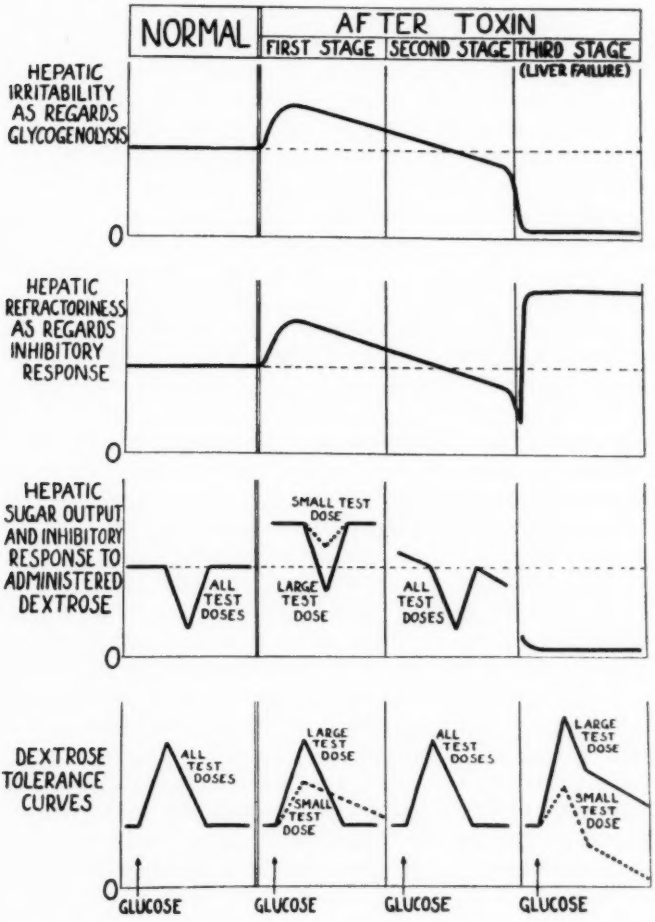

Fig. 4. Schematic representation of the progressive change in liver response to administered sugar, following toxin administration. It should be noted that the four items which are charted do not represent different processes, but are rather four different aspects of the same phenomenon.

In this connection it should be pointed out that in using a test dose of 0.25 gram glucose per kgm. body weight every hour, we were administering just about that amount of sugar which Mann and Magath (9) have shown can be disposed of by the completely hepatectomized dog.

SUMMARY AND DISCUSSION. A consideration of all the results obtained with the various doses of sugar used reveals 3 stages in the progressive change in the liver response to the administered sugar, following toxin administration; and indicates the important influence of the amount of sugar used for the test on the character of all 3 stages. In figure 4 we have attempted a schematic representation of these phenomena.

Stage 1 is characterized by an early decline in the inhibitory effect exerted by the administered sugar, so that the liver fails to decrease its own supply of blood sugar as rapidly as normal, and a "diabetic" type of tolerance curve results. This refractoriness of the liver to a stimulus to which it would normally respond is apparently related to the strength of the stimulus, since with larger doses of sugar the resistance may be overcome and normal curves obtained, while with smaller amounts of sugar the "diabetic" tendency becomes more and more apparent.

Stage 2 shows a return of the hepatic response towards the normal so that normal tolerance curves again appear. It is difficult to estimate how much of this effect, with a test dose of 0.25 gram sugar per kgm. (fig. 3), is due to an increasing responsiveness on the part of the liver and how much is due to a summation of inadequate stimuli, since the repeated administration of this small amount of sugar caused a steady rise in the basic blood sugar level during *stage 1* which may finally have represented a sufficient stimulus to the liver to cause it to give the normal inhibitory response, in spite of some remaining refractoriness. That *stage 2* is real and not an artefact, however, is apparent from experiments with larger amount of sugar, such as illustrated in figure 2, where the initial "diabetic" tolerance curve gives way to the normal, which persists until the final stage is reached, in spite of a falling base-line of blood sugar level.

Stage 3 consists of a more or less complete failure of hepatic function so that the effects obtained correspond closely to those observed in the liverless animal. Since the extrahepatic tissues can dispose of approximately 0.25 gram dextrose per kgm. per hour, the administration of less than this amount of sugar yields curves simulating a "supernormal" hepatic response, while the administration of greater amounts of sugar leads to an accumulation in the blood simulating a markedly "diabetic" type of curve.[4]

[4] A more detailed study of all our data seems to indicate that, between this final stage and the preceding stage of normal response, there is a transitory phase in which the liver retains a large part of its function but reacts in an abnormal measure to sugar administration so that truly "supernormal" responses are obtained.

In a previous report we have referred to the work of others which indicates that the first effect of a poison on the liver is to act as an irritant to the glycogenolytic mechanisms. We pointed out that it seemed reasonable that such a hyperirritability of the liver cells as regards the pouring out of sugar into the blood should limit the extent to which a given rise in blood sugar would inhibit this process. As the effects of the toxin on the liver progress to the point of mortal damage to the hepatic cells, the latter must pass from the state of glycogenolytic hyperirritability, through normal irritability, to hypoirritability and death. Translated into terms of the inhibitory reaction which determines the character of the dextrose tolerance curve, this cycle of events would be: First, a decreased inhibition of glycogenolysis yielding "diabetic" tolerance curves, unless the strength of the stimulus as represented by the administered sugar be great enough to overcome the refractory state of the organ, when a normal inhibitory response and therefore a normal tolerance curve may be obtained. Second, a return to the normal inhibitory reaction yielding apparently normal curves. Third, an increased inhibition of glycogenolysis yielding "supernormal" curves. This corresponds to the transitory phase between stages 2 and 3 mentioned in the footnote on page 654. And finally, a cessation of sugar output from the liver yielding the effects obtained in the hepatectomized animal. Our present data are in accord with the above conception.

It is clear from our results that a supposedly normal dextrose tolerance curve may, under appropriate circumstances, represent a greater degree of liver damage than a "diabetic" curve, and lesser degree of injury than a "supernormal" curve. This may account for the unsatisfactory nature of the attempts, mentioned in our introductory remarks, to correlate the results of dextrose tolerance tests with the clinical or pathologic criteria of liver damage. Such curves can be more correctly interpreted in the light of the cycle of events which we have demonstrated, and in conjunction with other evidence as to the extent and duration of the hepatic impairment. It is evident that a series of dextrose tolerance tests, performed at intervals during the course of a hepatic impairment, should yield information of greater prognostic value than could possibly be derived from any single test.

It is also possible that a comparison of tolerance curves obtained with large and small doses of sugar might be of clinical value since in stage 1 the large dose yields more normal curves than does the small dose, while in stage 3 the reverse is true.

CONCLUSION

1. The sequence of dextrose tolerance curves obtained by repeated tests during a progressively increasing toxemic liver damage, is related to the

nature and degree of the interference with the homeostatic mechanism by which the liver normally decreases its output of blood sugar in response to the influx of exogenous sugar.

2. The cycle of events following the onset of the toxemia depends on the amount of sugar used for the test. It is not a progression from the normal to increasingly abnormal curve, but passes from the normal to the "diabetic" type, then back to the normal, and again to the abnormal which may now be either "supernormal" or markedly "diabetic."

3. The relation of these results to the interpretation of the dextrose tolerance test is briefly discussed.

We wish to express our appreciation for the technical assistance of Mr. Jack Tarshis.

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LYMPH FORMATION DURING GLANDULAR ACTIVITY

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In addition to the long recognized importance of motion as affecting the rate of lymph flow, many authors ascribe great importance to osmotic changes in the tissue spaces as a factor in the increase of lymph flow observed during periods of activity.

This concept is based upon the work by Asher and Barbèra (1898) who reported an increase in the rate of lymph flow from the cervical lymphatic vessels after stimulating the salivary glands by placing blotting paper soaked with vinegar upon the tongue of a dog. Bainbridge (1900–1901) elaborated the above work, and also found an increase in the rate of lymph flow when the submaxillary gland was stimulated electrically through the chorda tympani and cervical sympathetic nerves or by pilocarpine injections. He later (1902) explained this increase during periods of glandular activity as possibly the result of a rapid formation of crystalloid metabolic products in the active gland cells; these metabolites entered the lymph spaces, probably by diffusion, and raised the osmotic pressure of the lymph, thus attracting water. An increase in lymph flow was the result.

Carlson, Greer and Becht (1907), in ten carefully performed experiments upon the submaxillary gland of the dog, could not confirm the work of Bainbridge nor could they obtain more lymph when the parotid gland of the horse was secreting actively. They offered no explanation for their negative results and their work remains unquoted.

Barcroft and Kato (1915–1916) approached the problem indirectly. They made no attempt to measure lymph flow. From the rise of hemoglobin in the blood as it traversed the actively secreting submaxillary gland of the dog, they calculated the fluid exudate from the blood during periods of activity and suggested that fluid unaccounted for as saliva was lymph. They concluded that increased functional activity occasioned an increased lymph flow.

Similar studies (Bainbridge, 1905; Falloise, 1903; Wertheimer, 1906; and Schmidt and Hayman, 1929–1930) on the liver, pancreas and kidney have been reported with conflicting conclusions. Most of this work may be criticized on account of the quantity and nature of the substances injected into the blood stream to induce glandular activity.

In view of the many contradictions in the literature upon this important subject and the improved methods of anesthesia and observation now available, it was thought the work of Bainbridge on the submaxillary gland should be repeated.

EXPERIMENTAL. Dogs ranging from 16 to 30 kgm. were anesthetized with nembutal intraperitoneally. The cervical lymphatic vessels were exposed and one cannulated on each side of the neck. The lymph vessels that drain the submaxillary gland lead in varying numbers from the hilus and enter a large lymph node which is situated near the bifurcation of the common carotid artery. From this lymph node one or two large vessels pass downward, closely paralleling the common carotid arteries, to terminate by entering the thoracic duct on the left and the subclavian vein on the right. If two of these vessels are present they have several cross communications. It is reasonable to suppose that if one of these vessels is cannulated and the other ligated as they enter either the thoracic duct or subclavian vein, the cannulated vessel will deliver the lymph produced in the area massaged in the head and neck region.

The lymph was allowed to flow into pipettes of 2 mm. diameter calibrated to 0.01 cc. The pipettes were placed in a horizontal position to avoid pressure changes. A small amount of heparin was placed in each cannula to prevent clotting. The average minute flow was calculated from readings made every five minutes except in a few experiments when minute readings were made. In order to secure lymph flow, regular massage (two vigorous strokes per minute) has been applied over the submaxillary gland and down each side of the neck throughout these experiments.

The relation of lymph flow to lymph formation. There is no flow of lymph or but slight flow from the cervical vessels in the quiescent animal until massage or motion is applied to the head and neck region. It is simpler to show the relation of lymph formation in the massaged area if the results are compared with the findings reported recently (White, Field and Drinker, 1933; and Weech, Goettsch and Reeves, 1934) on the rates of lymph flow from the extremities of dogs.

When the figures presented by these authors are charted (fig. 1), it is observed that there is no lymph flow during periods of rest. When motion (walking) is begun in the normal dog there is an initial rapid increase in lymph flow which falls quickly to a lower level and is there maintained as long as walking is continued. A slight increase was observed when activity was increased (running) but this was small when compared with the initial rise.

Weech, Goettsch and Reeves show this even more strikingly in their studies on dogs rendered edematous by plasmapheresis or by low protein diet. They report, "With the edematous dog the situation is similar but because the interstitial spaces contain more fluid (edema) the initial rapid

flow can be maintained for a longer time than in the normal animal. Within 10 or 15 minutes, however, the rate of flow decreases and continued activity is accompanied by progressive and finally by complete loss of edema." When the lower constant level was reached it was only slightly higher in these abnormal animals than the values observed by White, Field and Drinker for normal animals.

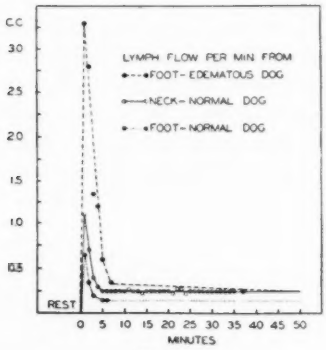

Fig. 1

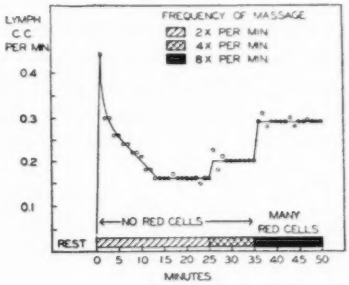

Fig. 2

Fig. 1. Lymph flow during walking or massage. *Curve* ●- - -● (foot—edematous dog), from Weech, Goettsch and Reeves, table 2. First minute flow calculated from first 42 seconds flow of table. *Curve* ●.....● (foot—normal dog), from White, Field and Drinker, table 4.

Fig. 2. Effect of increasing rates of massage over submaxillary gland on lymph flow from cervical vessels.

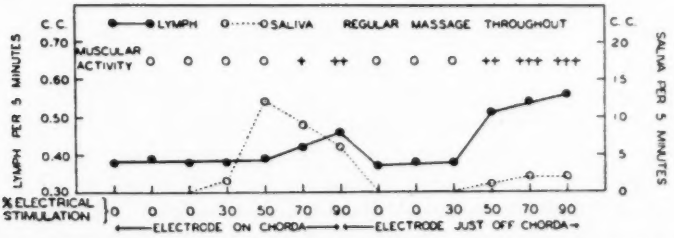

Fig. 3. Effect of increasing electrical stimulation on lymph and salivary flow.

When massage is applied over the submaxillary gland and down each side of the neck at regular intervals, the same typical rates of flow are observed from the cervical vessels. The variations that occur when the frequency of massage is increased is best shown in figure 2. Here again the increased massage, as increased activity, caused a rise in the lower constant level but it is small when compared with the initial rise. Very

frequent massage causes capillary damage, as is evident by the increase in the concentration of proteins and the appearance of erythrocytes in the lymph. But in our experiments, when too severe massage has been avoided, it is believed capillary permeability remained unaltered since the concentration of lymph proteins reached a low constant level simultaneously with the low constant level of lymph flow. Furthermore, no erythrocytes were observed in the lymph.

It appears obvious, as pointed out by Weech, Goettsch and Reeves, that the initial rapid rise in lymph flow represents the fluid of the interstitial spaces and the lower constant level is equivalent to capillary filtration or lymph formation. Activity, active or passive, supplies motion to force the tissue fluid and capillary filtrate into and along the lymph channels.

RESULTS. Table 1 shows the results of six experiments where electrical stimuli were used to activate the submaxillary gland. Lymph and salivary

TABLE 1

NUMBER OF EXPERIMENT	BEFORE SUBMAXILLARY ACTIVITY			DURING SUBMAXILLARY ACTIVITY			DURING SUBMAXILLARY ACTIVITY PLUS MUSCULAR CONTRACTIONS		
	Lymph flow	Salivary flow	Observation	Lymph flow	Salivary flow	Observation	Lymph flow	Salivary flow	Observation
	cc. per min.		*min.*	*cc. per min.*		*min.*	*cc. per min.*		*min.*
1	0.027	None	30	0.030	1.20	30			
2	0.026	None	40	0.023	1.30	30			
3	0.080	None	35	0.100	1.70	20			
4	0.101	None	45	0.080	1.07	10			
5	0.033	None	35	0.034	0.90	10	0.063	1.39	10
6	0.073	None	20	0.077	1.90	10	0.107	0.78	10
Averages...	0.051	None		0.057	1.34		0.850	1.08	

flow are recorded from one side only—that in which glandular activity was produced—since the opposite (control) side showed no variation from the normal. The only suggestion of an increase in the rate of lymph flow was in experiment 3. Here, due to a small incision made to expose the chorda, it was found difficult to adjust the current to the point where a copious flow of saliva was obtained without causing small muscular tremors at the same time. In view of this observation and because Bainbridge states in his original report that the variations in the rate of lymph flow were mainly dependent upon the strength of current used and only roughly correlated with the rate of salivary flow, stronger currents were used in the last two experiments. Muscular twitchings that occurred with the stronger current were roughly estimated by + for small tremors, + + + for rather violent contractions, and + + as midway between these two extremes.

As observed by Bainbridge, the increasing strength of current did increase the rate of lymph flow, but this increase occurred only with increasing muscular motion and appeared in no way related to salivary flow. The same results were obtained if the electrode was moved just off the chorda into the surrounding soft tissue (fig. 3).

Four experiments were then performed where pilocarpine hydrochloride in varying amounts (0.025 to 0.10 mgm. per kgm. of body weight) was given intravenously to stimulate salivary flow. It was found that even the smaller doses caused marked respiratory and vascular changes without any appreciable flow of saliva. Doses sufficient to cause a copious flow of saliva caused such respiratory embarrassment that the accessory muscles of respiration in the neck region became very active. The results were too confusing to permit discussion or tabulations.

SUMMARY AND CONCLUSIONS

In six experiments it has been impossible to confirm the reported increase in lymph flow from the cervical vessels after stimulating the submaxillary gland electrically except when the current used was sufficiently strong to cause simultaneous muscular activity.

Pilocarpine in amounts sufficient to cause a copious flow of saliva produced such marked respiratory and vascular changes that the data were unreliable as related to glandular activity and lymph flow.

These results are in entire agreement with the work of Carlson, Greer and Becht who in similar direct measurements failed to find that glandular activity increases lymph production and lymph flow.

The author is indebted to Dr. Cecil K. Drinker for suggesting this work and for his advice and assistance during this investigation.

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THE INFLUENCE OF HYPERPNEA AND OF VARIATIONS IN THE O_2 AND CO_2 TENSION IN THE INSPIRED AIR UPON NYSTAGMUS

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In the two preceding papers of this series, the effects of the three factors mentioned in the title have been studied in regard to some visual and auditory processes, in order to investigate fundamental conditions of cortical excitability. The question now arises whether the results obtained in those experiments are applicable to the brain as a whole or are characteristic of the cortex. In order to decide this question experiments were undertaken in which the effects of O_2-lack, CO_2-excess and hyperpnea were studied on a typical brain stem reflex, caloric nystagmus. Magnus (1924) has shown that nystagmus is present after the removal of the cerebellum. Furthermore, according to Kubo (1906) and Bauer and Leidler (1912), even the removal of the tel- and diencephalon does not interfere with the occurrence of caloric nystagmus (for further details compare Spiegel, 1931).

METHOD. We used the method of Veits (1928) by which even weak stimuli cause nystagmus. Five cubic centimeters of water of a temperature varying between 28° and 32°C. were injected through an ear funnel toward the posterior upper wall of the external auditory meatus. The temperature and speed of the flow of the water were rigidly controlled so that the injection lasted exactly ten seconds. During the injection, and for 48 seconds afterward the experimental subject was seated on a Barany chair, leaning his forehead against a support which kept the head bent forward 30°. After 48 seconds the head was bent backward by the experimenter in 2 seconds, so that the head rested in a position which was 90° different from the preceding one. The experimental subject directed his eyes toward the left and kept them in this position while the experimenter counted the number of nystagmic movements through a Frenzel lens. The nystagmus was recorded by the experimenter with a key and signal magnet on a kymograph. The stimuli are so mild that the experimental subject has no other sensation than that of slight coolness in the ear during the injection. In no case was vertigo observed. If controls are carried

out at intervals of 30 minutes and more, they show very great consistency; in fact the records taken over a period of weeks seem to indicate that under well-controlled conditions the number of nystagmi is very constant for each individual. The latent period of the nystagmus was also studied but did not show any significant changes. This is probably due to the fact that the nystagmus occurred with a very short latency immediately after the subject has taken the correct position and moved the eyes to the left.

RESULTS. The experiments on the influence of O_2-lack, CO_2-excess and hyperpnea on nystagmus are based on 325 observations on 6 trained subjects. Ninety-six preliminary tests were made in order to establish the technique. Hereafter 25 experiments with hyperpnea, 18 with CO_2-excess and 18 with O_2-lack were performed, which, due to the fact that each experiment was preceded and followed by one to two controls, comprised a total of 229 tests.

In the first group of experments the influence of CO_2-excess was studied. Five per cent to 7 per cent CO_2-air-mixtures were inhaled from a Douglas bag for 4 to 7 minutes. Immediately thereafter the caloric stimulation was performed. Figure 1 shows that the number of nystagmi is decreased immediately after CO_2 administration. Frequently there is no essential difference in the time during which nystagmi are observed, but the rate of nystagmic movements is considerably slowed. From the graphs of figure 1, it is apparent that the same person shows only slight differences in the controls, although the individual experiments were separated by several days and sometimes weeks.

The second group of experiments concerns the influence of hyperpnea. The experimental subject breathes maximally at a rate of 35 per minute for 2 or 3 minutes. Immediately thereafter the nystagmus was investigated. Also, in this case the effect was clear cut, showing a regular increase in the number of nystagmic movements in all experiments (fig. 2). The rate of nystagmi was not significantly altered.

In contrast to the two groups of experiments described above it may be stated that it was very difficult to get clear results with experiments on the effects of O_2-lack on nystagmus. In the first group of six experiments, $9\frac{1}{2}$ to $10\frac{1}{2}$ per cent O_2-N_2-mixtures were inhaled from a Douglas bag from 7 to 12 minutes. In 4 of these experiments the nystagmus was unaltered; in 1 a slight increase was observed and in 1 a decrease. Obviously, the degree of anoxemia produced by the inhalation of O_2 in the concentrations mentioned does not lead to any impairment in the function of the brain stem, although it causes considerable changes in hearing and vision. We tried, therefore, to produce an anoxemia of a considerably longer duration or intensity. Nine experiments of this type were carried out on six persons (table 1). In these experiments the experimental sub-

ject inhaled O_2-N_2-mixture of 8 per cent to 10 per cent from 2 to 3 Douglas bags, for as long as 50 minutes. In 6 experiments (on 5 experimental subjects) a decrease in the number of nystagmi was observed, which was

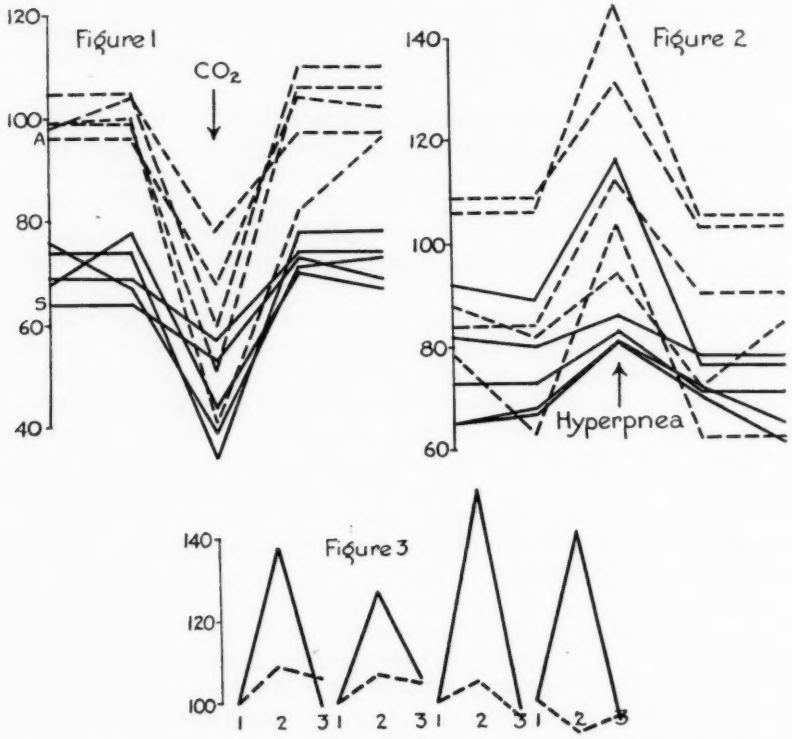

Fig. 1. The influence of CO_2 on the number of nystagmic movements after caloric stimulation. The CO_2-experiment was preceded and followed by two control experiments in which the experimental subject breathed air. The interrupted lines represent experiments with A. J.; the solid lines those with L. S.

Fig. 2. The influence of hyperpnea on the number of nystagmic movements.

Fig. 3. The influence of time on the effect of hyperpnea in regard to nystagmus. Experiments with 4 subjects; 1 and 3 controls, 2 after hyperpnea. Solid lines: caloric stimulation carried out immediately after hyperpnea; interrupted lines: caloric stimulation carried out 3 minutes after the end of the hyperpnea period.

completely reversible when, after thirty minutes of atmospheric air, a control stimulation was performed (nos. 1–6 in table 1). But even in these successful experiments the effect (with the exception of no. 5) was very

slight, compared with the considerable changes obtained by CO_2-excess and hyperpnea.

The experiments done with Mr. M. (nos. 7–9) are particularly interesting. This young man of an unusual physical fitness inhaled 10 per cent O_2 for as long as 50 minutes without experiencing any discomfort. However, in experiments on the latent period, duration, and intensity of after-images under the influence of O_2-lack, he showed the typical effects described in the preceding papers after inhalation of O_2 for as short a time as 6 or 7 minutes. Moreover, he gave the typical results with CO_2-excess and hyperpnea on nystagmus. In a final experiment he was subjected to an O_2-N_2-mixture of 8 per cent (no. 9). The experiment had to be discontinued after 6.2 minutes because of the severe symptoms. The face was extremely pale, the fingernails blue, he was nauseated and had the

TABLE 1

The influence of O_2-lack on nystagmus

NUMBER	EXPERIMENTAL SUBJECT	NUMBER OF NYSTAGMI			O_2-CONCENTRATION	DURATION OF O_2-LACK
		1. Control before O_2-lack	2. Immediately after O_2-lack	3. Control after O_2-lack		
					per cent	*minutes*
1	St.	92	72	95	10.1	19
2	J.	105	94	109	9.9	15
3	K.	114	91	103	10.0	25
4	Ma.	87	69	90	10.5	12.5
5	Ma.	88	39		10.1	25.5
6	Kan.	106	94	109	10.0	22.5
7	Me.	92	93	90	9.87	30.0
8	Me.	97	98		10.5	50.0
9	Me.	97	96	106	8.0	6.3

feeling of faintness. But the caloric stimulation performed immediately after the O_2-lack period had been discontinued did not show any change at all.

Since in the previous papers it has been shown that the effects of hyperpnea and O_2-lack lasted considerably longer than the duration of the experiment, the question was investigated whether such after-effects could be found in brain stem experiments. Since the O_2-lack effect on nystagmus was too small and since the CO_2-effect was temporary even in experiments with vision and hearing, we decided to study this question in regard to the effect of hyperpnea on nystagmus. The experiments were run in the usual manner, except for the fact that the caloric stimulation was not made immediately after the hyperpnea period was over, but three minutes later. All experiments had the same result. The number of nystagmi was the

same as in the controls, indicating that the effect of hyperpnea on nystagmus had completely ceased in this short interval (fig. 3).

Discussion. The outstanding difference in the reaction of the brain stem investigated by the nystagmus reflex, and of the sensory cortex, studied in regard to auditory threshold and visual after-images in the preceding papers, is the fact that the effect of O_2-lack is very profound on cortical phenomena and comparatively small on a function involving subcortical structures. This observation is in agreement with those of other authors (literature by Gildea and Cobb, 1930) according to which the cortex of the brain is most sensitive to O_2-lack, whereas brain stem and spinal cord show a considerably less degree of sensitivity. Our experiments show that similar relations hold true for the human brain. If conditions are chosen with prolonged O_2-lack, the nystagmus decreases. The phenomenon seems to indicate a decrease of excitability of the nervous mechanism involved, which is comparable but quantitatively different from similar changes in the excitability of the cortex.

The effect of CO_2-excess resulting in a diminution of the number of nystagmic movements seems also to harmonize with the decreased excitability of the cortex under similar conditions. There is, however, a fundamental difference between cortex and brain stem in their reactivity to hyperpnea. Whereas hyperpnea leads to a decrease in cortical excitability, it increases the brain stem reflex (nystagmus) considerably. The brain stem reacts as does the spinal cord, since King, Garrey and Bryan (in animals) and Strughold and Jörg (in man) have found that hyperpnea increases tendon reflexes. The discrepancy of the results obtained with the cortex and with the brain stem seems to be explainable on the basis of the assumption of a differential sensitivity to O_2-lack. As has been discussed in detail in the preceding papers, we know, on the basis of Cobb's and Schmidt's experiments, that hyperpnea leads to a vasoconstriction. The effect of hyperpnea on nervous tissue is the resultant of two antagonistic factors: First, of the specific increase in excitability of the nervous tissue whenever the CO_2-tension in the blood is decreased in hyperpnea, and, second, of the vasoconstriction which diminishes the O_2-supply to the tissues. If the sensitivity of the nervous tissue to the latter factor is very great, as is the case with the cortex, the vasoconstriction may outweigh the other factor, and as a result, we have a decrease in excitability. If, however, the O_2-sensitivity is not great, as has been shown to be true for the brain stem, the specific effect of the decreased CO_2-tension on the nervous tissue must result in an increased excitability.

Taking into account the great differences in sensitivity of various parts of the central nervous system to O_2-lack, we come to an understanding even of their qualitatively different reactions on a uniform basis. It seems, however, not unimportant to stress the significant differences at various

levels of nervous activity. Here should be mentioned the fact that after-effects are very marked in regard to auditory and visual processes after O_2-lack or hyperpnea, but cannot be detected in similar experiments on nystagmus. This difference must be due either to differences in the sense organs or in the central nervous mechanisms involved. Whereas it is very difficult and uncertain to separate in vision and hearing peripheral processes in the sense organs from those occurring in the cortex following the stimulation of the former, such an attempt may be successful in regard to the effects of vestibular stimulation. Mowrer (1935) has recently shown that the electric discharges of the vestibular nerve resulting from rotation cease completely immediately after the cessation of the stimulus (rotation). The nystagmus must, therefore, be due to an after-discharge of the central neurons. This interpretation agrees well with findings of Fischer and Oldberg (1932) who emphasize the importance of the central neurons for the intensity of the nystagmus reaction. In the light of these observations it is very probable that O_2-lack, CO_2-excess and hyperpnea chiefly affect the central neurons. The great difference in sensitivity to O_2-lack in the various reactions studied allows us to specify the location of the important neurons or synapes still further: They are probably cortical in the cases of vision and hearing and subcortical in nystagmus. We come to the conclusion that besides the qualitatively different reaction between cortex and subcortex to hyperpnea, there are considerable quantitative differences not only in regard to the immediate effect of O_2-lack but also in regard to its after-effects, which are more marked in the cortex. It is, moreover, not improbable that the differing effects of hyperpnea on the several processes are due in the last analysis to the different reactions of the respective neurons to O_2-lack, which plays an indirect rôle in hyperpnea because of resultant vasoconstriction (compare the first paper of this series). The CO_2-effect is found to be the same for the brain stem as it is for the cortex. Here we are concerned with the effect of the changes in CO_2-tension on the excitability of nervous tissue. These changes, of course, lead to alterations in hydrogen ion concentration, but we have not attempted to differentiate effects of each, if indeed the CO_2-tension has any effect excepting that through the changes in pH, which in turn affects such factors as the ionization of calcium.

SUMMARY

In studies on the effect of CO_2-excess, O_2-lack and hyperpnea on nystagmus in the human, it was found that breathing CO_2-air-mixtures results in a decrease in the number of nystagmic movements and that hyperpnea produces an increase in this number. The brain stem reacts to increases and decreases in the CO_2-tension of the blood with the same direction of change in excitability as occurs with spinal reflexes. O_2-lack produces

an effect on the nystagmus (decrease in number) only under the conditions of severe anoxemia. The effects are temporary, and as soon as 3 minutes afterwards control values are obtained. These reactions are strikingly different from those observed in the case of the more strictly cortical processes reported in the preceding papers. The differences appear to be largely associated with the relative sensitivity to O_2-lack. The cortical neurones are extremely sensitive to alterations in oxygen supply, while the brain stem centers studied, either because of a more adequate blood supply, or for other reasons, are relatively insensitive to such changes.

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THE INFLUENCE OF SODIUM TAUROCHOLATE, HEPATIC BILE AND GALL-BLADDER BILE UPON THE ABSORPTION OF OLEIC ACID FROM THE SMALL INTESTINE[1]

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This communication is a report of experiments made to determine the effect of sodium taurocholate, of hepatic bile and of gall-bladder bile upon the absorption of oleic acid from isolated jejunal loops prepared in dogs by the method of Johnston (1). Intestinal loops so made permit a more accurate quantitative determination of absorption from the small intestine than was possible in the few earlier reports dealing with this problem (Plant, 2; Verzar and Kuthy, 3). In the experiments here reported it was found that the absorption of oleic acid, negligible when this substance was introduced alone, was greatly increased in the presence of sodium taurocholate, slightly less so in the presence of hepatic or of gall-bladder bile.

Experiments were made upon 4 dogs. A constant quantity of oleic acid, 869 mgm., was introduced into the loops alone or with either sodium taurocholate, hepatic bile or gall-bladder bile. The amount of sodium taurocholate employed varied from 82 to 246 mgm. Normal dog's bile, hepatic or gall-bladder, was varied in amount so that the taurocholate content of the bile introduced was between 166 and 275 mgm. Tap water was used to bring to 30 cc. the total volume of fluid introduced in each experiment. At the beginning of each experiment, therefore, the oleic acid concentration was 0.102 mole per liter, while the taurocholate varied from 0.005 to 0.016 mole per liter.

Oleic acid was introduced into and removed immediately from the loops in a series of control observations upon each dog. In all other experiments the loops were closed for 1, 2 or 3 hours after which they were emptied, thoroughly washed with water and the washings added to the material originally recovered. This material was quantitatively analyzed for its oleic acid content. The fatty acid was saponified with potassium hydroxide, acidified with hydrochloric acid and extracted with petroleum ether. Aliquot portions of the petroleum ether extract were evaporated to dryness,

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taken up in 95 per cent alcohol and titrated with standard sodium hydroxide. This method permits recovery of 95 per cent of a known quantity of oleic acid added to intestinal juice in vitro. Intestinal juice alone thus tested contained a small amount of material reacting as fatty acid. Since the amount present was relatively constant and did not exceed 3 per cent of the total oleic acid used in the experiments it has been disregarded. The material withdrawn after introduction of bile salt or bile

TABLE 1

Per cent of oleic acid not recovered from intestinal loops when introduced alone or in combination with sodium taurocholate, hepatic bile or gall-bladder bile

MATERIALS USED	MEAN PER CENT LOSS				
	Dog 1	Dog 2	Dog 3	Dog 4	Average all experiments
Removed immediately					
Oleic acid (869 mgm. used in all experiments)	5.9 ±0.8* (6)†	12.4 ±2.1 (4)	11.2 ±2.6 (10)	6.6 ±1.5 (6)	9.1 ±1.2 (26)
Removed after 2 hours					
Oleic acid	15.4 ±1.4 (12)	10.6 ±2.0 (7)	12.2 ±6.5 (4)	10.9 ±1.9 (6)	12.9 ±3.7 (29)
Oleic acid + sodium taurocholate (82–246 mgm.)	45.6 ±2.0 (21)	36.7 ±3.6 (5)	21.8 ±1.2 (7)	28.4 ±3.5 (4)	38.0 ±2.1 (37)
Oleic acid + hepatic bile (taurocholate content: 166–275 mgm.)	41.9 ±4.1 (5)	19.0 (1)	13.0 ±3.5 (5)		26.7 ±5.0 (11)
Oleic acid + gall-bladder bile (taurocholate content 166–275 mgm.)	35.7 ±2.2 (7)	23.0 ±3.9 (6)	16.6 ±2.3 (6)		25.7 ±2.4 (19)

* S.E. of mean = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† = Number of experiments entering into the mean.

was analysed also for taurocholate but the method of analysis on mixtures containing intestinal juice was subject to large errors and, therefore, quantitative figures are not given.

From the results presented in table 1 it will be seen that:

1. The dogs often differ significantly from one another in the degree of their response to a given procedure. The averaging together of the experiments on different dogs is, therefore, open to criticism.

2. Comparison of the data obtained when oleic acid was removed imme-

diately with those secured after oleic acid alone had been in the loop 2 hours, shows that in dogs 2 and 3 the percentage of oleic acid lost is not significantly different in the two periods and there is, therefore, no definite evidence of absorption when oleic acid alone was used. In dogs 1 and 4 slight absorption of oleic acid when introduced alone cannot be denied but the evidence that it occurred is not convincing.

TABLE 2

Absorption of oleic acid (869 mgm. introduced) under different conditions, and after varying intervals in dog 1

OLEIC ACID		OLEIC ACID + HEPATIC BILE		OLEIC ACID + GALL BLADDER BILE	
Immediate withdrawal	2 hours (12 observations)	Removed after 2 hours		Removed after 2 hours	
Lost	Lost	Sodium taurocholate introduced	Oleic acid lost	Sodium taurocholate introduced	Oleic acid lost
per cent	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
6.3	9.0 to 26.4	165	53	166	35
9.3		165	43	166	30
4.6	Av. = 15.5	255	30	174	36
5.5		255	37	210	48
4.0	S.D. 4.7†			275	33
5.5					

OLEIC ACID + SODIUM TAUROCHOLATE

Removed after 1 hour		Removed after 2 hours			Removed after 3 hours	
Sodium taurocholate introduced	Oleic acid lost	Number of experiments	Sodium taurocholate introduced	Oleic acid lost	Sodium taurocholate introduced	Oleic acid lost
mgm.	*per cent*		*mgm.*	*per cent*	*mgm.*	*per cent*
165	25	6	82	52 ±3*	165	36
165	24	4	100–110	44 ±3	165	39
165	28	4	166–186	40 ±4	165	50
		3	246	40 ±6		

* S.E. of mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† S.D. of observations $= \sqrt{\frac{\Sigma d^2}{n-1}}$

3. Comparing the influence of hepatic and gall-bladder bile on absorption of oleic acid, in none of the dogs is there a significant difference in the effect of the two types of bile when the total bile salt content is about the same. In dog 3 absorption is not significantly demonstrated in either instance; in dog 2 absorption is definite though small and in dog 1 it is marked.

4. In the experiments with sodium taurocholate all the dogs exhibit the

same order of increased absorption, this being definitely greater than with bile although in dog 1 the difference is hardly significant.

From table 2 it is evident that:

1. There is no correlation between the amount of sodium taurocholate in excess of 82 mgm. introduced and the per cent of oleic acid absorbed.

2. The absorption of oleic acid continues for at least two hours but the absorption in 3 hours is not demonstrably greater than at the end of 2 hours.

The relative concentrations of oleic acid and taurocholate in the fluid introduced into the loops preclude any direct chemical reaction between the two as an explanation for the absorption of oleic acid. During the periods of closure of the loop, fluid was poured into the loop in varying quantities. At the end of 1 hour the average quantity of fluid withdrawn was 62 cc., the concentration of oleic acid 0.038 mole per liter and approximately half of the bile salt had been absorbed. The fact that absorption proceeded for an additional hour indicates that even a low concentration of taurocholate was effective during the second hour. At the end of 3 hours taurocholate if present was found in only very small amounts and no evidence was obtained that fat was absorbed during this period. No correlation was found between the amount of fluid withdrawn from the loop either with or without taurocholate and the total quantity of oleic acid absorbed. It is apparent, therefore, that the action of taurocholate in facilitating the absorption of oleic acid must be in the nature of a surface phenomenon and that very minute concentrations are sufficient to produce absorption of relatively large quantities of fatty acid.

CONCLUSIONS

From the foregoing experiments it is concluded that the absorption of oleic acid is negligible when introduced alone into a loop of small intestine, is greatly increased by the presence in the loop of sodium taurocholate, and somewhat less so by hepatic bile or gall-bladder bile.

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FURTHER STUDIES OF THE EFFECTS OF THE ESTROGENIC AND THE GALACTOPOIETIC HORMONES UPON THE MAMMARY GLAND OF THE RABBIT

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The influence of the estrogenic hormone (ovarian follicular hormone or theelin) upon the stimulation of mammary growth has been extensively investigated in several species of laboratory animals (Turner et al., 1930–1934). The normal development of the mammary glands of these species have also been studied (Turner et al. as cited above). Theelin induced a growth of the mammary duct system but did not induce an appreciable development of the alveoli, except in the guinea pig. The type of development of the mammary glands induced by theelin was comparable to the normal development observed in post-pubertal virgin animals.

The hormone of the corpus luteum was necessary to produce the further development of the mammary glands (Turner et al., 1931–1934). The experimental development of the glands may thus be divided into a stage of duct development induced by theelin and a stage of alveolar development requiring the hormone of the corpus luteum in addition to theelin. In the normally developing mammary glands the sudden development of the alveolar elements occurred during pseudo-pregnancy or during the first half of pregnancy (fig. 4).

Lactation was not observed following the administration of the ovarian hormones. Stricker and Grueter (1928) first demonstrated that a preparation of the anterior pituitary induced lactation. This observation has been verified and extended (Corner, 1930; Asdell, 1931; Turner and Gardner, 1931; Nelson and Pfiffner, 1931; Catchpole and Lyons, 1933; Gardner and Turner, 1933; Riddle, Bates and Dykshorn, 1933; Lyons and Catchpole, 1933; Donahue, 1934).

Corner (1930) was of the opinion that the pituitary would also induce growth of the mammary glands. Asdell (1931) observed that immature female rabbits did not respond to a similar pituitary preparation. Lyons and Catchpole (1933) reported obtaining lactation and mammary growth

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in mature castrate virgin rabbits. In contrast to the above observers, Gardner and Turner (1933) concluded that the pituitary did not stimulate a real growth of the mammary glands but acted on the existing epithelium stimulating secretory activity. The involuted glands of old castrate or the glands of ovariectomized immature rabbits were not developed or were not stimulated to secretory activity by galactin except in one rabbit when theelin preceded galactin treatment. The enlargement of the mammary glands following such treatment was the result of distention with secretion rather than a true growth stimulation. The marked thickening of the mammary glands observed during normal lactation was never observed in such animals without the previous existence of the alveoli.

The present investigation was undertaken to further study the specificity of the anterior pituitary galactopoietic hormone and the effect of this hormone on the mammary gland duct system.

MATERIALS AND TECHNIQUE. Immature and mature castrate virgin female, castrate multiparous, and normal and castrate male rabbits were used in the following experiments. Thus the effect of the galactopoietic hormone was investigated upon the partially developed mammary glands of young and mature male and female rabbits.

A total of 47 experiments were performed using 43 rabbits. The duration and rate of theelin[2] injections varied in different experiments as will be indicated later. The amounts of lactation stimulating hormone used were tested and known to contain in excess of one rabbit unit.

The developmental changes of the glands induced by theelin injections were determined either on the removed glands or by observations at laparotomy. The removed glands were studied either as whole gland preparations or were sectioned for histological examination, or both. The degree of response to the lactation stimulating hormone was determined by observation. Histological preparations of such glands were used to further portray their structure.

Galactin treatment following the ovariectomy of young virgin rabbits. Four rabbits were used in this series. Though estrus was not tested by attempted mating in two animals it is probable they were all in estrus at the time they were ovariectomized. The mammary glands removed at the time of ovariectomy consisted of an extensive duct system (figs. 1 to 3). A typical gland removed from one of the rabbits at this time measured 3 × 5 cm.

Three rabbits received galactin during the 6 or 7 days immediately

[2] The estrogenic hormone used was theelin. This was kindly supplied by Parke Davis Co. The galactopoietic hormone (galactin) was largely prepared by modifications of methods previously described by the writers. Four male rabbits received "Prolactin." This extract was kindly supplied by Dr. J. F. Anderson, of E. R. Squibb and Sons.

following ovariectomy. The mammary glands of one injected and of the uninjected rabbit were examined on the third day and found to contain a serous secretion. On the day following the cessation of injections the mammary glands of the injected rabbits were all distended with a milk-like fluid. The main ducts were enlarged and together with the dilated small lateral ducts and sprouts gave the gross impression of a fully lactating gland. Though some cellular hyperplasia may have occurred it had not been extensive enough to change the type of mammary structure which was still essentially a compound tubular gland. The mammary glands of the uninjected control rabbit still contained some clear fluid on the seventh day.

This experiment confirms in part the observations of Corner (1930), Asdell (1931) and Catchpole and Lyons (1933). The effect of the lactation stimulating hormone, however, was assigned to a mammary hypertrophy resulting from the accumulation of secretion rather than to both a growth and a secretory stimulation.

The effect of galactin following theelin injections on the mammary glands of immature ovariectomized rabbits. It has been previously demonstrated (Asdell, 1931; Gardner and Turner, 1933) that the mammary glands of immature female rabbits do not respond to galactin. The mammary glands of such rabbits consisted of duct systems extending less than 1 or 2 cm. beyond the base of the nipple. Though the ovaries of such rabbits may possess follicles their hormone production must be quite limited as the mammary glands develop slowly during the pre-pubertal period. If the growth of the mammary glands were suddenly accelerated would they respond to galactin?

Fourteen immature female rabbits were used. Twelve of the animals were from 3 to 4 months old while two were somewhat older. The ovaries of the twelve younger rabbits contained no large follicles at the time of ovariectomy. Several moderately large follicles were observed in the ovaries of the two older rabbits. The control mammary glands removed from the younger rabbits extended but slightly beyond the base of the nipple. Twenty and 25 rat units of theelin were given daily for 20 days starting immediately after ovariectomy. Examination of the mammary glands following theelin injections showed that a marked growth had been induced. The glands measured from 2 to 6 cm. in diameter. In a few cases some serous fluid was present in the ducts.

An excess of one rabbit unit of galactin was given during 6 and 7 days for the younger and older rabbits, respectively. The mammary glands were again examined the day following the cessation of galactin treatment. Again there was no evidence of growth of the mammary glands following the discontinuance of the theelin injections. In all instances the ducts were distended with milk or a milk-like secretion. The results were es-

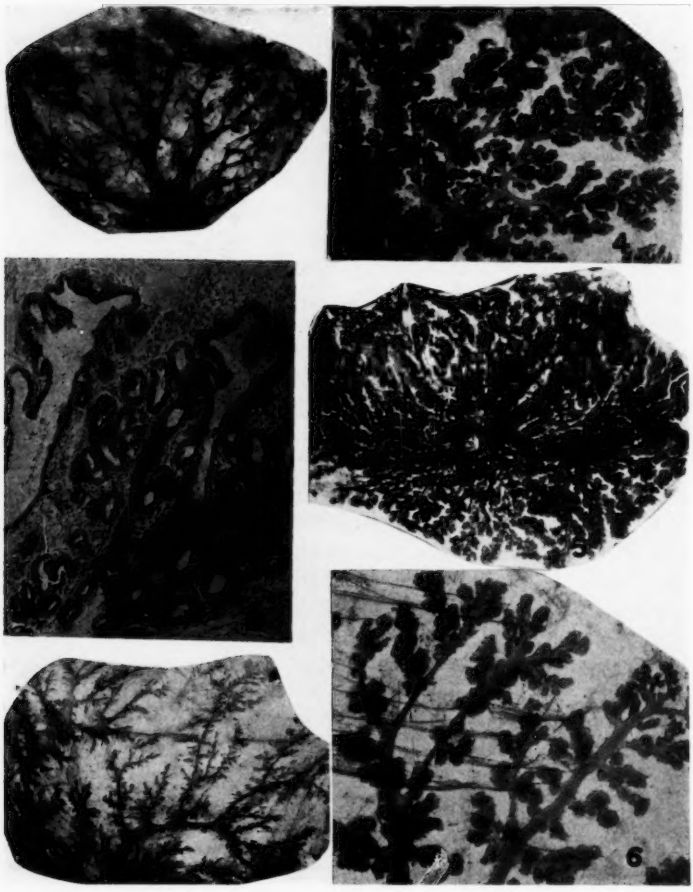

Plate 1. Fig. 1. Microphotograph of a portion of the mammary gland of a sexually mature nulliparous rabbit. The gland was removed immediately after copulation. × 4.

Fig. 2. Microphotograph of portion of the sectioned mammary gland shown in figure 1. × 6.5.

Fig. 3. Microphotograph of a portion of the mammary gland of an adult nulliparous rabbit during anestrum. × 2.6.

Fig. 4. Microphotograph of a portion of the mammary gland of a rabbit at the end of pseudo-pregnancy (15 days). × 2.6.

Fig. 5. Microphotograph of a whole mount of the mammary gland of the rabbit at the time of parturition. The milk was expressed from the gland before fixation and staining. See figure 7 for section. × .65.

Fig. 6. Microphotograph of a portion of the mammary gland of a sexually mature

nulliparous rabbit which received 20 rat units of theelin daily for 20 days followed by 2 cc. of galactin administered once daily for six days. Note the secretion in the lumina of the large ducts and slight hypertrophy of the lateral and peripheral outgrowths. See figure 9 for section. × 2.6.

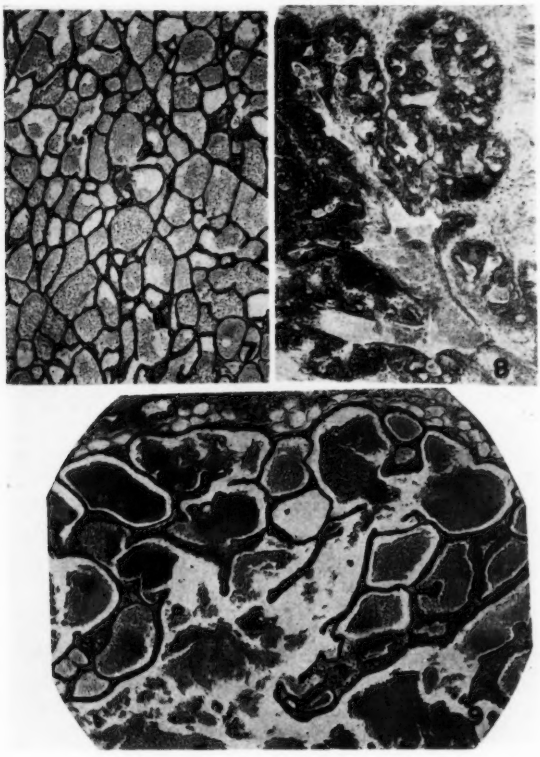

Plate II. Fig. 7. Section of lactating rabbit mammary gland. Lactation was induced by galactin at the end of pseudo-pregnancy. × 19.5.

Fig. 8. Section of the mammary gland of a young male rabbit which received 20 rat units of theelin for 20 days followed by 2 cc. of galactin daily for six days. Note the secretion in the lumina of the ducts and the multilayered epithelium of the walls of the lateral branches. × 19.5.

Fig. 9. Section of the lactating mammary gland of the sexually mature female rabbit shown in figure 6. Note dilatation of the duct end buds in comparison with the alveoli of a fully grown (lobular-alveolar system) and lactating mammary gland. × 19.5.

sentially similar to the previous series except that the amount of distention of the glands was not as marked as in the proceeding instance. Whereas the extent of lactation was rated as plus two (++) in the previous experiment it was rated as plus one (+) in the present series.

The effect of galactin following theelin upon the regressed mammary glands of ovariectomized multiparous rabbits. Following ovariectomy the mammary glands of multiparous female rabbits undergo an extensive involution. Within a few months only the duct system remains and this also slowly regresses so that finally only the larger mammary ducts persist. From the viewpoint of mammary gland structure, the regressed mammary glands of ovariectomized multiparous rabbits are comparable with those of mature virgin females (Turner, 1932; Gardner and Turner, 1933).

Eleven multiparous rabbits whose mammary glands had been allowed to regress for at least 100 days were used in the present series of experiments. Four of these rabbits were given daily injections of 20 rat units of an oil solution of theelin for 10 days followed by one rabbit unit of galactin during six days. Secretion was not observed in the mammary gland ducts following the theelin injections and in the mammary glands of only one rabbit following the galactin injections. The amount of secretion in this case was very slight.

A second series of six similar rabbits was similarly treated except the theelin injections were continued for 20 days and two of the rabbits received 25 rat units of water soluble hormone daily. The mammary ducts of the two rabbits receiving the 25 rat units of theelin contained a small amount of a serous secretion the day the injections were stopped. Following the injection of one rabbit unit of galactin daily during six days the mammary ducts of all the rabbits were distended with the contained milk (figs. 6 and 9).

One similar rabbit received galactin without previous theelin treatment. Secretion was not observed in this animal.

As in the two preceding series of experiments galactin was observed to be effective in inducing lactation after the duct system had been under the influence of theelin. Also, as in the previous series, there was no evidence that the galactopoietic factor induced the growth of the glands. The changes in the glands were the result of the distention of the mammary ducts. The ten day period of theelin injections was concluded to be too brief to sufficiently repair or condition the glands to respond to galactin.

The effect of galactin following theelin on the mammary glands of normal and castrate male rabbits. The rudimentary mammary glands of nine male rabbits, five of which were normal and four of which were castrated, were developed by theelin stimulation. The experiments were repeated in two of the normal males a second time so a total of 11 experiments were performed.

One normal and one castrate male were treated with 20 rat units of theelin for ten days. The mammary glands at this time were slightly developed. Galactin was then injected for six days. The mammary glands showed no evidence of further growth after the cessation of theelin injection. Again the ten day periods of injections were not sufficiently long to develop or condition the glands for any secretory response to galactin.

Seven of the rabbits received 20 or 25 rat units of theelin daily for 20 days. Two of the latter rabbits were later given 50 rat units of theelin daily for 14 days. The mammary duct system developed under the influence of theelin extended from 2 to 3 cm. out from the base of the nipple. In all cases the ducts were quite large and in some instances they were observed to contain a serous fluid.

Galactin (prolactin was given to four of the rabbits in this series) was given the rabbits immediately following the cessation of theelin treatment. After treatment for six to seven days definite secretory activity was observed in all cases. In some of the rabbits this consisted of the presence of milk or a milk-like fluid in the gland ducts (fig. 8). In other rabbits the ducts were greatly distended with milk.

The further growth of the mammary glands during the period of galactin or prolactin treatment was not observed. Likewise the extent of lactation observed never equaled that observed following the injection of galactin into male rabbits whose mammary lobules and alveoli had been developed in addition to the mammary ducts, by the simultaneous injections of theelin and progestin or corporin.

The effect of delaying galactin treatment following theelin. Is it necessary to start the injection of the galactopoietic hormone immediately following the cessation of theelin or will the mammary glands remain sensitive to galactin for a period of time?

Five multiparous female rabbits whose mammary glands were involuted for over 100 days were injected for 20 days with 20 rat units of theelin daily. One rabbit received galactin for six days beginning on the second day after theelin treatment was stopped, one on the third, and one on the fourth, etc. Lactation was induced in the rabbit in which galactin treatment was started on the second day following theelin injections. The mammary glands of the rabbit given galactin on the third day following the theelin treatment showed some indications of secretory activity following the galactin treatment. None of the other rabbits gave any indications of response.

Though this series of animals is very small it is indicated that the mammary glands rapidly regress to a point where they will not respond to galactin following the cessation of theelin treatment.

Re-initiation of lactation by galactin following theelin. It has been ob-

served that galactin would not induce a second lactation response in castrate female rabbits following an experimentally stimulated lactation. Normal female rabbits also failed to respond a second time until the ovaries had again been allowed to act upon the mammary glands (Gardner and Turner, 1933). The possibility that theelin might be effective in the development or conditioning of the glands to galactin following a previous experimentally induced lactation was investigated in two mature, ovariectomized, multiparous rabbits. Ten days after the onset of previous experimentally induced lactation 20 rat units of theelin were injected daily for 20 days. At the time theelin injections stopped the glands consisted of a well developed duct system. Some lobules were observed near the nipples. After injecting galactin for six days the entire mammary structure was again engorged with milk.

Discussion. The normal development and function of the mammary glands has been experimentally reproduced in immaturely ovariectomized or male rabbits (Turner and Frank, 1932; Gardner and Turner, 1933); guinea pigs (Nelson and Pfiffner, 1931; Nelson and Smelser, 1933; Turner and Gomez, 1934); and cats (Turner and DeMoss, 1934). Thus far the guinea pig is the only species extensively investigated in which the hormone of the corpus luteum has not been required in the stimulation of complete mammary gland growth. Theelin administration for as long as 120 days has not been observed to produce a development of the mammary glands of rabbits beyond the duct stage, whereas theelin and corporin injected simultaneously produced complete mammary growth (Turner and Frank, 1932). A similar limitation of theelin stimulation during long time experiments has also been observed in mice (Turner and Gomez, 1934; Gardner et al., 1934). Corporin alone was not effective in the stimulation of the mammary growth in the rabbit (Corner, 1930; Turner and Frank, 1931) but when given simultaneously with the theelin induced a complete mammary development (Turner and Frank, 1931, 1932). The primary ovarian follicular hormone and corporin (progestin) thus induced the stimulation of complete mammary growth. Mammary growth is essentially under ovarian control as indicated by Ancel and Bouin (1911). There appears to be no experimental evidence for the statement of Evans (1935) concerning the "non-necessity of progestin in mammary physiology." The specificity of response of the mammary glands to the ovarian follicular[3] and luteal hormones is as definite as is the specificity of response of the uterus to the same hormones, particularly in the rabbit.

[3] The writers are aware that "Theelin," the purified commercial hormone used may not be the same chemical substance found in the ovary. At this time the similarity is based on biological rather than chemical data. The biological similarity, however, is considered to justify the reference to theelin as an ovarian follicular hormone.

Asdell and Seidenstein (1935) observed that the mammary glands of hypophysectomized rabbits could be developed by the stimulus of the ovarian hormones, theelin and progestin. Jeffers (1935) also observed the further development of the mammary glands of rats hypophysectomized during pregnancy The growth and initial secretory processes of the mammary glands of such rats progressed normally. The above observations indicate that the pituitary is not essential for normal mammary gland growth except indirectly through the ovary.

No evidence of mammary gland growth was observed following the cessation of theelin injections in the above experiments. Though the mammary glands were denser and sometimes were actually of somewhat greater extent it appeared that this was due essentially to a distention of the duct system with secretion. Examination of the glands removed before and after galactin injections fail to show an increase in number of the smaller ducts (figs. 1–6). Likewise, it is only rarely that a lobular structure is found following galactin injections when none were observed in the control tissue previously examined. Though there may be some cellular hyperplasia it is certainly of secondary importance and is not sufficient to change the glands from the theelin induced compound tubular structures (figs. 8–9). The mammary gland duct epithelium undoubtedly possesses secretory capacities. Weatherford (1929) noted histological evidence of secretory activity of the smaller ducts in normally lactating rats; that is, in animals with fully developed mammary glands. This might be expected as the mammary alveoli develop as small out-growths from the smaller ducts. Histologically the epithelium of the smaller ducts and alveoli are identical. The smaller ducts apparently function both in supplying more secretory epithelium and in conducting the secretion from the terminal structures.

In the normal animal the proper physiological condition for the activation of the secretory epithelium of the mammary glands is not reached until the complete or almost complete development of the mammary glands has been induced. Mammary secretory activity is not observed normally except following pregnancy and, in some species, as in the dog, and ferret, following pseudo-pregnancy (figs. 5 and 7). During pregnancy the physiological conditions are such that mammary gland growth is produced.

The observations of Corner (1930) and Asdell (1931) showed that lactation resulted in mature ovariectomized virgin rabbits following the administration of lactation stimulating hormone. Such rabbits, though they have extensively developed mammary duct systems, possess very few if any mammary alveoli. Since estrus is more or less continuous in the isolated mature virgin rabbit it is probable that the mammary glands were influenced optimally by the animal's own ovarian secretions up to

the time of ovariectomy. When such rabbits were treated with the lactation stimulating hormone immediately following ovariectomy it was probable that the glands were subjected to the influence of lactation stimulating hormone immediately following theelin. In other words, under certain conditions the existing epithelium of the partially developed mammary gland may be stimulated to functional activity.

The observations reported in the present paper verify the above conclusions. The mammary gland ducts of male rabbits grown during theelin treatment responded to the lactation stimulating hormone. Similar results were observed when galactin was given old rabbits whose duct systems had been partially restored following extensive castrate atrophy. The mammary ducts of ovariectomized immature rabbits also responded to galactin after their slow development had been accelerated by theelin.

The influence of theelin also appears to be required for a certain definite period before the mammary epithelium will respond to galactin. None of the rabbits responded when theelin had been injected for a period of only 10 days. The glands also appeared to become refractory to galactin very soon after theelin injections were stopped. The small series of experiments reported above gave completely negative results following the lapse of more than three days between the cessation of theelin treatment and the beginning of galactin treatment.

What effect theelin has upon the mammary duct epithelium in conditioning its response to the galactopoietic substance is not known. A serous fluid has been observed quite frequently in the ducts following theelin injections alone but this fluid is definitely distinct from colostrum or milk and is present only in relatively small quantities. Only after the administration of galactin has the presence of a substance grossly and microscopically resembling milk been observed.

As theelin does in some way prepare the partially developed mammary glands for response to galactin it might also be assumed to similarly prepare the fully developed glands.

SUMMARY

The mammary duct systems of ovariectomized immature rabbits and of male rabbits developed under the influence of theelin respond to the galactopoietic hormone of the pituitary. Likewise, the mammary duct systems persisting after previous ovariectomy of multiparous females respond to galactin after theelin stimulation.

The galactopoietic hormone produced no definite growth of the mammary glands. The mammary glands of all the rabbits consisting of duct systems prior to galactin injections consisted essentially of a duct system following the injections. The increased density and area of the glands

were attributed primarily to a distention of the preëxisting ducts with fluid.

A period of theelin treatment in excess of ten days was required before the mammary glands of rabbits, not in estrus or recently ovariectomized during estrus, would respond to galactin. Indications of secretory activation of the mammary glands were observed in all rabbits treated with galactin immediately following a previous 20 day period of theelin treatment.

It is indicated that the mammary glands of the theelin injected rabbits fail to secrete when galactin treatment is delayed for more than three days after the cessation of theelin injections.

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A STUDY OF THE COMPARATIVE PHYSIOLOGY OF THE GLOSSOPHARYNGEAL NERVE—RESPIRATORY REFLEX IN THE RABBIT, CAT AND DOG

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During an investigation of "A pharyngeal inspiratory reflex of the cat" (11), our interest was aroused by an apparent variation in the reflex respiratory responses of certain laboratory animals. In the text-books compiled by Luciani (5), Stewart (10), Greene (2), Macleod (6), Howell (3), and Evans and Hartridge (1), it is stated that respiration is reflexly inhibited by stimulation of the central end of the glossopharyngeal nerve. Of those who investigated the rôle of the glossopharyngeal nerve in respiration, Schiff (9) noted that it causes a prolongation of inspiration in the rabbit; Knoll (4) produced marked inspiratory changes; and Markwald (7) obtained complete inhibition of respiration in the same animal. The authors (11) have been able to confirm Markwald's observations in the rabbit.

MATERIAL AND METHOD. Fourteen cats, 10 dogs and 6 rabbits were used in the experiments to be reported. Barbital-Na was administered intravenously under ether, the dose being 275 mgm. per kilo. The ether was then discontinued. Faradic current of varying intensities was employed. Respiration was recorded by means of a tambour connected to a tracheal cannula.

RESULTS. *Rabbit.* Stimulation of the central end of the glossopharyngeal nerve causes inhibition of respiration in this animal, as is evident in a typical record illustrated in figure 1. The carotid sinus branch of the glossopharyngeal nerve may, however, cause an acceleration of respiration, as in figure 2. The response recorded in figure 1 confirms the earlier observations of Markwald (7). The inhibition, which may occur in either inspiration or expiration, has a duration of 2 to 3 respirations; and then respiration breaks through regardless of the prolongation of the stimulus.

Cat. Of the 14 cats, only 1 responded to stimulation of the central end of the glossopharyngeal nerve in a manner comparable to that obtained in the rabbit (fig. 3). In two other cats some inhibition was elicited, but

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the response was not comparable to that of the rabbit; for it was interrupted by respiratory acceleration, as may be the case in the dog.

The reflex respiratory response to glossopharyngeal nerve stimulation in the cat is usually initiated by a deep inspiration, as depicted in figure 4. This inspiration may be followed by a more or less prolonged expiration which may be interpreted as a phase of incomplete inhibition. Following the prolonged expiration which may be interrupted by shallow, rapid gasps, there may be a short inhibitory phase which is followed by marked acceleration of respiration; or more frequently there is an immediate marked respiratory augmentation as in figure 4.

The accelerator response in the cat is primarily inspiratory in nature in most instances. While the rare inhibitory response, as in the case of the

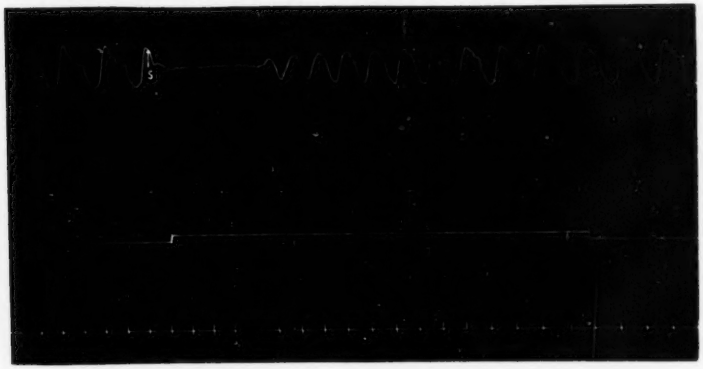

Fig. 1. Rabbit. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 5 volts, secondary coil at 9 cm. Time, 1 second.

rabbit, has a short duration despite the prolongation of the stimulus, the more common accelerator response lasts as long as the stimulus is continued; being interrupted however by the element of fatigue if continued long enough. From the above it is quite evident that the anoxemia resulting from the respiratory inhibition is capable of overcoming the reflex nervous apnea despite the continuation of the electrical stimulus.

Dog. The respiratory response to central glossopharyngeal nerve stimulation is less constant in the dog than it is in the cat and rabbit. Of the 10 dogs studied, 7 responded with respiratory acceleration. Two of these gave a marked response, as illustrated in figure 5, on stimulation of both the right and left glossopharyngeal nerves. In a third dog the left nerve caused moderate acceleration, while the right caused slight inhibition of respiration. The 4 other animals, in addition to the accelerator response,

also evinced an inhibitory mechanism which involved either different branches of the same nerve, or different phases of the response to the stimulation of an individual nerve, as in figure 6.

Of the 10 dogs, 8 responded to central glossopharyngeal nerve stimulation with varying degrees of inhibition. The latter, as illustrated in

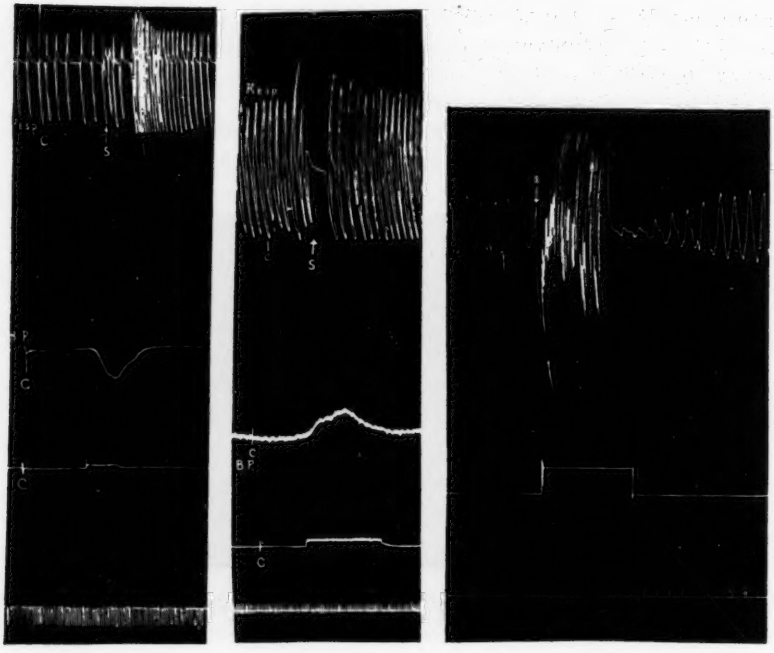

Fig. 2 Fig. 3 Fig. 4

Fig. 2. Rabbit. Stimulation of the central end of the carotid sinus branch of the right glossopharyngeal nerve. Primary, 5 volts, secondary coil at 11 cm. Time, 1 second.

Fig. 3. Cat. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 6 volts, secondary coil at 10 cm. Time, 1 second.

Fig. 4. Cat. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 6 volts, secondary coil at 12 cm. Time, 3 seconds.

figure 7, resembled that occurring in the rabbit in some cases. In others it was less marked. In 4 of the animals an accelerator response was also present, as in figure 6. In dogs in which the inhibitory response is not evident, as in the case illustrated in figure 5, it cannot be claimed that the mechanism involved is altogether absent; for the nature of the distribution

of the fibers might be such, that the accelerator response conceals the one of inhibition. That the above is true was evident in several dogs in which certain branches caused inhibition and others acceleration; but the main trunk produced acceleration alone.

DISCUSSION. The present-day text-book conception that the glossopharyngeal nerve reflexly inhibits respiration has most likely been derived from Markwald's (7) description of the response obtained in the rabbit. In addition, Markwald correlated his findings with the observations of Meltzer (8), who came to the conclusion that respiration is inhibited during deglutition, on the basis of experiments performed upon himself. This correlation led Markwald to believe that deglutition inhibits respiration through the activity of the glossopharyngeal nerve. One of us has repeated Meltzer's experiments on himself, but could not confirm his findings. In a number of experiments carried out on dogs, the details of which shall be presented in a later publication, we could not confirm the generally accepted opinion that deglutition inhibits respiration through the glossopharyngeal nerve; for the effect of deglutition on respiration, which was a momentary inhibition, remained unaltered after both glossopharyngeal nerves were cut. On the basis of the observations reported above for the cat, it is also difficult to reconcile oneself to respiratory inhibition as the result of deglutition through the medium of the glossopharyngeal nerve. We did observe, however, that the glossopharyngeal nerve has an extremely marked inhibitory effect on the act of deglutition, as elicited by central stimulation of the superior laryngeal nerve.

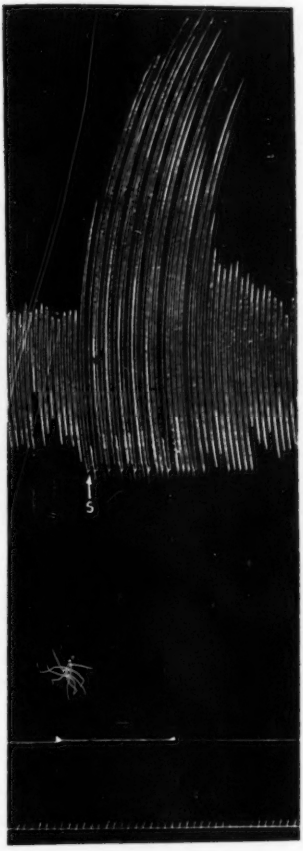

Fig. 5. Dog. Stimulation of the central end of the right glossopharyngeal nerve. Primary, 4.5 volts, secondary coil at 10 cm. Time, 1 second.

SUMMARY

In the rabbit, stimulation of the central end of the glossopharyngeal nerve (with exception of the carotid sinus branch which may cause acceleration) gives rise to respiratory inhibition. In the cat the response is usually

one of respiratory acceleration. More rarely inhibition, as in the case of the rabbit, is elicitable. In the dog the response is more variable, for a fair proportion of these animals respond with both inhibition and acceleration; while more respond with inhibition alone than with solely acceleration.

A preliminary series of experiments indicates that section of the glossopharyngeal nerves does not influence the effect produced by deglutition on respiration; but glossopharyngeal nerve section does markedly increase the number of swallowing movements elicited by stimulation of the central end of the superior laryngeal nerve.

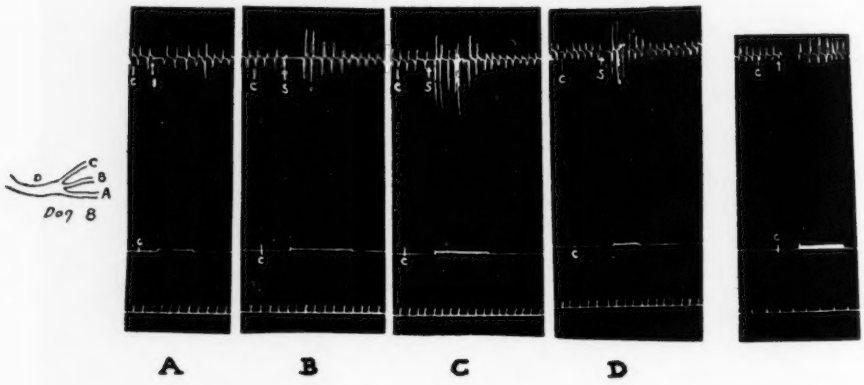

Fig. 6 Fig. 7

Fig. 6. Dog. A, B, and C, stimulation of the central end of branches A, B, and C of the left glossopharyngeal nerve. D, stimulation of the central end of the left glossopharyngeal nerve. Primary, 6 volts, secondary coil at 10 cm. Time, 1 second.

Fig. 7. Dog. Stimulation of the central end of the left glossopharyngeal nerve. Primary 5 volts. Secondary coil at 10 cm. Time, 1 second.

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THE COÖPERATIVE ACTION OF SYMPATHETIC NERVE IMPULSES, ADRENINE AND SYMPATHIN ON THE NICTITATING MEMBRANE OF THE CAT

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The coöperation between sympathetic impulses and adrenine, although long claimed to be possible (Elliott, 1912; Cannon, 1914), is supported by fragmentary experimental facts only. Besides the work of Elliott (*loc. cit.*) on blood pressure, some evidence is afforded by recent investigations on the nictitating membrane (n.m.) of the cat. Injected adrenine was found to intensify the direct nervous effect (Rosenblueth and Rioch, 1933a), and secreted adrenine increased the effect of sympathin—a humoral product of sympathetic nervous activity—from the lower abdominal chains (Rosenblueth and Cannon, 1932). Furthermore, sympathin from the heart reinforced that from the splanchnic area (Rosenblueth and Morison, 1934).

The studies mentioned, while suggestive of probable coöperation of the different sympathetic components in the organism, are not exhaustive. Moreover, no combinations of more than two sympathetic components have thus far been demonstrated. To supply such information, the present work was undertaken.

METHOD. Cats were used under dial ("Ciba," 0.8 cc. per kgm. intraperitoneally) or urethane (25 per cent, 6 cc. per kgm. intravenously). Isotonic contractions of the n.m.—either normal or denervated by resecting the corresponding superior cervical sympathetic ganglion two weeks previously—were recorded as described by Rosenblueth and Cannon (*loc. cit.*) with 2 to 4 grams of tension from a lever affording an 18-fold magnification. Shielded electrodes were used for stimulation. The duration of each stimulation was limited to a half minute. The stimulators were two Harvard induction coils and a neon tube unit. A possible mutual enhancing influence of the stimulators on each other when simultaneously applied was controlled.

Stimulation of the peripheral end of the cut cervical sympathetic supplied nerve impulses to the n.m. Adrenal secretion was elicited by direct

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excitation of a splanchnic nerve or reflexly from afferent activation of a sciatic nerve. Sympathin was obtained from the effectors supplied by the cardio-accelerator nerves, the hepatic nerves, and the lower abdominal sympathetic chains. Whenever a nerve to a certain organ or part was stimulated, its connections other than the one desired were sectioned if possible. The adrenals, when their secretion was not under investigation, were always removed.

Before the removal of the adrenals, cocaine (8 mgm. per kgm. intravenously) was injected to enhance the n.m. response. When reflex adrenal secretion was required, cocaine was omitted, since it abolishes the reflex. Curare was used, in addition to the anesthetic, to immobilize the animal whenever necessary. To avoid asphyxia, artificial respiration was employed.

RESULTS. 1. *Preliminary observations.* The n.m. response remained practically unchanged when a stimulus of a given intensity was repeatedly applied to the cervical sympathetic, but decreased progressively with chemical stimuli—adrenine or sympathin—under constant conditions (cf. Rosenblueth and Morison, *loc. cit.*). The rate of decrease of the chemical responses appeared to be related to the interval between stimulations and to the intensity of the stimulus. Thus the decrease in the responses was more marked with strong stimuli applied frequently. Only in rare cases was such a decrease not apparent.

The response to chemical stimulation differed from the nervous one in having a longer latency and a slower rate of contraction and relaxation. The following reasons may account for these differences. The nerve impulses are delivered to the n.m. directly, but the chemical agent does not become effective until an adequate concentration is reached in the blood stream. The localized nervous effects are readily dissipated, while the generalized chemical ones wear off but gradually.

The continuous rise of the n.m. and the delayed relaxation after strong nervous stimulation, reported by Rosenblueth (1932b), was also noted. The present observations, however, were made in cocainized animals. From the following data we believe that sympathin from effectors other than those in the n.m. was superimposed on the nervous effect in our case. Bacq (1933) showed that enough sympathin could be produced from a peripheral cervical sympathetic nerve to affect the retractor penis. We found that sympathin from the contralateral cervical sympathetic could occasionally cause a cocainized n.m. contraction. Sympathin from such a source, usually too little to act even on a cocainized n.m. by itself, readily called forth a bigger n.m. response when superimposed on a nervous effect (fig. 1).

From the above data, together with the fact that the n.m. responses increase asymptotically with respect to the strength of stimulation (Rosen-

blueth et al., 1932a, b, 1933b, 1934), it is clearly seen that, for such a study as this, submaximal stimulation should be used. First of all, submaximal stimulation will produce a practically pure nervous response which will be submaximal. Secondly, a submaximal response will allow further contraction of the n.m. in response to an added chemical influence. Finally, the chemical effect will not diminish so rapidly with submaximal stimulation.

2. *Summation of stimuli.* When stimulation of the cervical sympathetic just failed to evoke a response from the n.m., a superimposed chemical stimulus following the first one at varying time-intervals produced no effect. The stimulus therefore probably failed to set up any nerve impulses in the cervical sympathetic. When two subminimal chemical stimuli—sympathin from two sources or sympathin and adrenine—were

Fig. 1

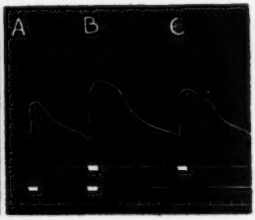

Fig. 2

Fig. 1. Dial, cocaine and curare. Upper signal: stimulation of the contralateral cervical sympathetic. Lower signal: stimulation of the ipsilateral cervical sympathetic. In this and the succeeding figures the time signal records 30-second intervals.

Fig. 2. Previously denervated nictitating membrane. Dial and cocaine. Upper signal: stimulation of right cardio-accelerators. Lower signal: stimulation of left cardio-accelerators.

produced simultaneously, contraction, which might have been undetectable with either one alone, appeared. In the latter case, the inadequate concentration of one chemical substance must be made adequate by the presence of the other. The coöperation between a subminimal chemical stimulus and a minimal (or submaximal) chemical or nervous one was readily demonstrated (fig. 1).

When two stimuli—either both minimal or one minimal and one submaximal—were superimposed, a greater response resulted (figs. 2B, 3C, E and G). The height of the resultant contraction was greater than the sum of the two separated contractions. The duration was usually prolonged. The summated response increased with increasing intensities of the stimuli until finally the resultant response became different from the individual ones by a longer duration only. A second stimulus also short-

ened the latency and hastened the rate of contraction. The form of the resultant contraction curve with a predominant nervous component exhibited a sharper transition from contraction to relaxation than that with a predominant chemical one (compare E with A and D, fig. 3). The greater of the two chemical influences appeared to determine the shape of the resultant contraction curve (contrast G with D and F, fig. 3).

The above holds true with three stimuli when the combination of any two is considered as one stimulus and the third as the other (figs. 3B and 4A). In so far as the contractions of the n.m. are concerned, all the

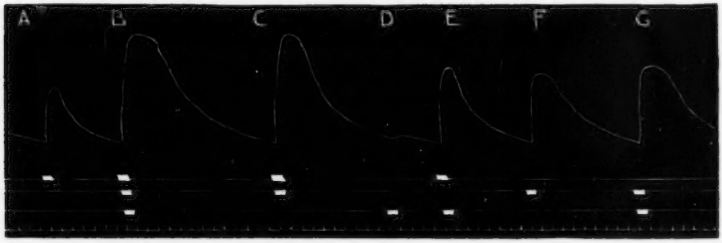

Fig. 3. Urethane and cocaine. Upper signal: ipsilateral cervical sympathetic. Middle signal: splanchnic supply to adrenal. Lower signal: hepatic nerves.

Fig. 4. Previously denervated nictitating membrane. Urethane and cocaine. Upper signal: splanchnic supply to the right adrenal gland. Middle signal: hepatic nerves. Lower signal: lower abdominal sympathetic chains.

effective stimuli have worked similarly. It is only on account of the different shapes of the responses that a distinction is made between the chemical and the nervous stimuli. The coöperation between different sympathetic components has been found demonstrable in any combination of two or three.

Discussion. Our findings support the conclusion that sympathetic impulses, adrenine and sympathin act synergetically (Cannon, 1934) on the n.m. and point to other interesting suggestions. The demonstration of subminimal chemical effects with the help of a minimal (or submaximal) chemical (or nervous) one (fig. 1) not only confirms the previous finding

of Rosenblueth and Cannon (*loc. cit.*) but also appears to be a delicate method for detection of small quantities of sympathin. If it can be proved to be true with sympathin I, which can be demonstrated in small quantities only, the method will be quite useful.

Since sympathin from stimulation of the contralateral cervical sympathetic sums in the cocainized cat with the effects on the n.m. of the ipsilateral nerves (fig. 1), it has been pointed out above that a similar summation probably occurs also of the sympathin liberated locally at the n.m. with that liberated at other effectors, e.g., pilomotors or vessels, when only one cervical trunk is stimulated. A similar summation may occur at any other effector observed, when the nerve stimulated supplies other structures. This is a possible source of error which should be kept in mind when it is necessary to eliminate the action of any other factor than the nerve impulses evoking a given response.

The fact that simultaneous bilateral stimulation of the cardio-accelerators produces a bigger response of the n.m. than either one alone (fig. 2) indicates that the number of nerve fibers activated determines the amounts of sympathin produced.

SUMMARY

Summation of different sympathetic components—direct sympathetic nerve impulses, sympathin from other sources, and adrenine—was studied by their simultaneous effects on the nictitating membrane of cocainized cats. A coöperation of these diverse stimuli was found as indicated by effective summation of all combinations of two or three tried (figs. 1, 2, 3 and 4). A subminimal chemical stimulus, e.g., sympathin, is capable of increasing the response to any of the other excitatory agents (fig. 1). The method, therefore, affords an opportunity to reveal minute amounts of sympathin, otherwise undetectable.

I wish to express my gratitude to Prof. W. B. Cannon for his suggestion of the problem and to Drs. H. Davis and A. Rosenblueth for their counsel.

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PHYSICAL ACTIVITY AND THE BLOOD OF ALBINO RATS[1]

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In this study a comparison has been made of the blood of exercised and sedentary white rats in regard to the number of erythrocytes, content of hemoglobin, and number of reticulocytes per unit volume of blood. An attempt has also been made to discover whether or not in active animals the body might compensate for the increase of erythrocyte destruction by the production of red cells of greater resistance to the agents of blood destruction.

Animals ten weeks old were divided into two groups—the exercise and control groups. Each group was fed a well-balanced diet, supplemented by fresh lettuce two or three times a week to insure a vitamin sufficiency. The rats of the first group were placed in individual cylindrical revolving activity cages six evenings each week at about five o'clock and were released at eight the following morning. The food trays were removed from both groups during the period of exertion of the active group. The exercised rats ran on an average of about four miles per night; the females ran consistently somewhat longer than the males. Some of the exercised animals were started in December and others on February 4 and continued on the program until May 15. The average gain in weight during the period of investigation was 93.8 grams for the control animals, and 127.5 grams for the exercised animals. Hence it is evident that the exercised animals were at least as healthy as the sedentary animals of the control group.

Contrary to expectation, it was found that there was no difference in the bloods of rats subjected to an exercise period of several months' duration and the bloods of litter mates kept sedentary for an equal period of time. The final condition of the blood of each rat in erythrocytes, hemoglobin, and reticulocytes is given in table 1.

From this table it is seen that the number of erythrocytes and content of hemoglobin are practically the same for all animals regardless of the fact that one group had experienced between four and five months of considerable daily physical activity; while the other group, because of the smallness of their cages, had been restricted to little or no physical exercise. Appar-

[1] This research has been conducted with aid of a grant from the Charles Himrod Denison Fund.

ently the increased demand for oxygen by the exercised group was not enough to call for the circulation of an additional supply of red corpuscles or for corpuscles richer in hemoglobin. This latter statement seems true, since both the content of hemoglobin and the number of erythrocytes is practically the same for all animals, thus giving a color index approximately equal for both groups.

It was possible that the exercise taken by the active group might cause increased blood destruction to such an extent that the bone marrow would be compelled to increase its production of erythrocytes in order to maintain the normal supply of corpuscles; hence the reticulocyte count, which determines the number of young red cells, may be of interest. A study of table 1 shows no significant difference between the two groups of animals; therefore we may assume that the amount of exercise taken by our active group did not stimulate the bone marrow.

TABLE 1

CONTROL RATS				EXERCISED RATS			
Animal no.	Red cells	Hemoglobin in 100 grams blood	Reticulocytes for 10,000 cells	Animal	Red cells	Hemoglobin in 100 grams blood	Reticulocytes for 10,000 cells
	millions				*millions*		
1	8.47	14.27	5	9	8.15	13.8	8
2	7.45	13.03	16	10	7.35	13.18	6
3	7.70	13.12	9	11	8.27	13.51	12
4	8.46	15.24	14	12	8.80	14.23	14
5	8.68	13.42	9	13	8.00	13.77	11
6	8.62	13.14	19	14	8.56	13.71	17
7	8.38	13.9	14	15	8.44	13.35	11
8	8.92	13.56	5				
Average...	8.34	13.71	11.25		8.22	13.65	11.29

In view of Broun's (1923) observation that a marked erythrocyte destruction occurs during vigorous exercise in dogs previously kept sedentary, we had reason to expect that we should find evidence in our exercised animals of either increased resistance of the red blood cells to wear and tear or stimulated bone marrow activity.

If compensation took the form of the production of erythrocytes of greater resistance to the agent of blood destruction, then methods that test cell fragility should reveal a difference between our two groups of animals. Two methods were employed to test resistance: first, different dilutions of saponin by Eric Ponder's (1923) method and, second, different concentrations of hypotonic saline by the method of Simmel (1923). By both methods it was found that there was no significant difference in the resistance to hemolysis of the corpuscles of the two groups of animals.

The bone marrow of the two groups of animals was next inspected to discover any difference in the activity of the blood forming tissue. The femurs of the animals were excised. One bone of each rat was split lengthwise and the halves examined with a binocular dissecting microscope. The extremities of the bone from the opposite leg of each rat were cut off and an hypodermic needle inserted into the lumen of the shaft. Then through the shaft were forced two cubic centimeters of a solution of brilliant cresol blue dye dissolved in isotonic saline. This solution was a second time forced through the shaft, but in the opposite direction. In this perfusing fluid the number of cells and of reticulocytes was carefully counted. In both groups of animals an average of about 57 reticulocytes to 500 assorted bone marrow cells was found. This similarity was confirmed by inspection of the marrow lying within the split bones. Neither quantitative nor

TABLE 2

Blood changes during a compulsory run

ANIMAL NUMBER	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
	millions	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
16	8.11	8.87	9.4	115	121	5.2
17	8.14	9.51	16.8	117	129	10.3
18	8.85	10.51	18.8	113	129	14.1
19	8.14	9.50	16.7	115	129	12.2
20	9.86	10.32	4.7	120	129	7.5
21	9.04	9.35	3.4	116	128	10.3
22	8.85	9.53	7.7	120	134	11.7
23	8.89	9.26	4.2	120	127	5.8

developmental differences between the two groups of animals could be observed.

Since in every erythrocyte study made by us no difference was found in the bloods of rats subjected to an exercise period of several months' duration and the bloods of litter mates kept sedentary for an equal period of time, we are led to conclude that a moderate amount of physical activity does not stimulate the blood-forming centers of the rat. We estimate that during the first seven weeks of exercise the average distance run by each animal was about 160 miles. Before the end of the period of observation each animal had run about four times that distance. This amount of voluntary activity compares favorably with that reported by Slonaker (1925).

The effect of a single period of vigorous exercise. It is well known that in man, the horse, the dog, and other mammals a short period of strenuous physical effort results in a temporary concentration in the number of erythrocytes and in the hemoglobin of the blood. The white rat likewise

develops an increase in the number of circulating erythrocytes during a run. This was demonstrated on a group of normal rats by exercising them in a treadmill driven by an electric motor. The animals were compelled to run rapidly for from two to three and one-half minutes, long enough to cause them to show signs of fatigue and respiratory distress. Blood counts and hemoglobin determinations were made before the exercise while the animals were at rest, and directly after the muscular exercise. Previous to the experimentation, the rats were tamed in order to reduce excitement upon being handled. The data for before and after exercise are given in table 2. It is to be noted that an increase in the number of circulating erythrocytes and the content of hemoglobin is displayed in every instance following the muscular activity. The average number of erythrocytes for the animals while at rest was 8,740,000, and after exercise was 9,606,000, which is an average increase of 9.9 per cent. The content of hemoglobin rose from an average of 117 (Haldane scale) during rest to 129 after exercise, or an average rise of 10.2 per cent.

In view of the discussion regarding the explanation of how the increase in erythrocytes is brought about during a short period of exertion, the spleens were excised from a number of rats. The operation caused them to become anemic; hence they were fed liver and iron compounds in addition to the regular diet. While they improved markedly within a period of two weeks, they were not back to normal when experimentation had to end. Among them were three animals that had returned to within 8 and 10 per cent of their normal blood composition. These, as well as others, failed to develop the usual increase in the number of circulating red blood cells when compelled to run in the treadmill. It would seem, therefore, that the spleen in the normal rat serves as an important storehouse of reserve erythrocytes and that these may be brought into active circulation during a period of strenuous physical activity.

SUMMARY

Regular moderate physical activity over a period of months does not stimulate the hematopoietic tissue of the white rat to increased activity. This was demonstrated by the failure of the erythrocytes, hemoglobin, and reticulocytes to increase in a unit volume of blood. The resistance of the red corpuscles to hemolysis was also not modified by regular exercise.

A brief period of strenuous running produced a temporary rise in the erythrocyte and hemoglobin content of the blood of normal rats but failed to do so in splenectomized animals.

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THE CARBON DIOXIDE CONTENT AND COMBINING POWER AND pH OF CERVICAL LYMPH

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Information concerning lymph gases is meagre. The few observations that do exist are well summarized by Drinker and Field (1933) and are principally concerned with the carbon dioxide content of thoracic duct lymph. Similar data for peripheral lymph are limited to the work of Tsuji (1934) who determined the carbon dioxide content of lymph in afferent and efferent vessels of the popliteal lymph node of rabbits. The data with respect to the hydrogen ion concentration of lymph are somewhat conflicting. von Krüger reports a pH value of 9 for thoracic duct lymph, while Gesell concludes that the blood is invariably more alkaline than the lymph. The only observations on peripheral lymph were made by Tsuji (1933) who reports values of 7.92 and 7.65 for lymph in the afferent and efferent vessels of the popliteal lymph nodes of rabbits. Details concerning the conditions under which the measurements were made are not given and corresponding values for simultaneously collected blood samples are not included for reference.

In view of the uncertain state of the literature with respect to the above mentioned properties of lymph, it was decided to investigate more thoroughly the carbon dioxide content and combining power and pH of cervical lymph of the dog. This lymph is easy to collect and can usually be obtained in sufficient quantity to provide duplicate or triplicate determinations for each observation.

TECHNIQUE. *Sampling.* Dogs weighing in excess of 20 kgm. were anesthetized with an intraperitoneal injection of nembutal. The cervical lymphatic trunks were exposed and cannulated. A small amount of heparin was placed in the tip of the cannulae to prevent coagulation. When analyzing for content, the lymph was collected anaerobically in specially constructed syringes (Behnke, Shaw, Shilling, Thomson and Messer, 1934) and approximately 1 cc. introduced directly into the chamber of the Van Slyke apparatus. Blood samples were withdrawn from the jugular vein by means of an oiled syringe and introduced under oil into the constricted centrifuge tubes of Myers and Muntwyler. Heparin was used as an anticoagulant. The blood was centrifuged and measured as described by Peters and Van Slyke (1932).

Methods. The carbon dioxide analyses of lymph and plasma were made by means of the Van Slyke manometric apparatus (Van Slyke and Neill, 1924) on 1 cc. samples. Duplicate or triplicate determinations were invariably made. Data for the dissociation curves were obtained by equilibrating the lymph and plasma in tonometer tubes with known tensions of carbon dioxide. The equilibration procedure was carried out for 20 minutes at 38°C. and the final composition of the gas mixture was determined by gas analysis. In the experiments in which the animal was permitted to breathe carbon dioxide, a gas mixture consisting of approximately 10 per cent carbon dioxide, 20 per cent oxygen, and 70 per cent nitrogen was

TABLE 1

The carbon dioxide combining power of separated plasma and cervical lymph

DOG NUMBER	SEPARATED PLASMA			CERVICAL LYMPH		
	CO_2 tension, mm.Hg		$\Delta CO_{2_{60-30}}$	CO_2 tension, mm.Hg		$\Delta CO_{2_{60-30}}$
	30	60		30	60	
	volumes per cent			*volumes per cent*		
1	53.6	59.8	6.2	59.1	62.8	3.7
2	50.4	55.3	4.9	51.6	56.6	5.0
3	56.5	62.8	6.3	57.3	61.9	4.6
4	56.2	62.3	6.1	59.4	63.7	4.3
5	56.5	61.8	5.3	61.8	65.3	3.5
6	58.1	63.5	5.4	57.5	61.8	4.3
7	59.2	65.0	5.8	60.5	66.2	5.7
8	50.8	56.0	5.2	52.5	57.3	4.8
9	54.0	59.0	5.0	57.2	61.5	4.3
10	62.3	68.4	6.1	66.8	71.5	4.7
11	51.2	56.1	4.9	54.1	59.5	5.4
12	61.6	67.5	5.9	63.6	68.4	4.8
13	63.5	68.2	4.7	66.2	70.3	4.1
14	53.4	57.4	4.0	56.6	60.5	3.9
17	54.0	59.5	5.5	60.1	64.5	4.4
Average..	56.1	61.5	5.4	58.9	63.4	4.5

administered through a tracheal cannula. Alveolar air samples were taken by means of the apparatus of Shaw and Messer (1930). The pH determination was conducted according to the method of Eisenman (1927) with the exception that the saturation technique of Austin was not used. The composition of the gas mixture in the tonometer tubes was determined by gas analysis. In order to apply this method to the determination of the lymph pH, the constants pK′ and α of the Henderson-Hasselbalch equation must be known for lymph. Peters and Van Slyke (1931) give identical values of pK′ for lymph and blood plasma. The solubility coefficient α of carbon dioxide in lymph is not known, but by inference from the work

of Van Slyke, Sendroy, Hastings and Neill (1928), we can safely assume that it does not differ greatly from that of plasma. These authors have shown that the deviation in the coefficient with respect to water and serum is principally due to the presence of three elements in the latter fluid: salts and protein which depress the solubility 3 and 2 per cent, respectively, and lipoids which increase the solubility about 4 per cent. The salt concentrations of plasma and lymph differ only slightly while the lymph has less than half as much protein and fat. From these relationships one can see that the solubility coefficient is approximately the same in lymph as in plasma. Furthermore, it can be shown that a variation of 5.7 per cent in the value of α causes an alteration in the pH of only 0.001, which is within

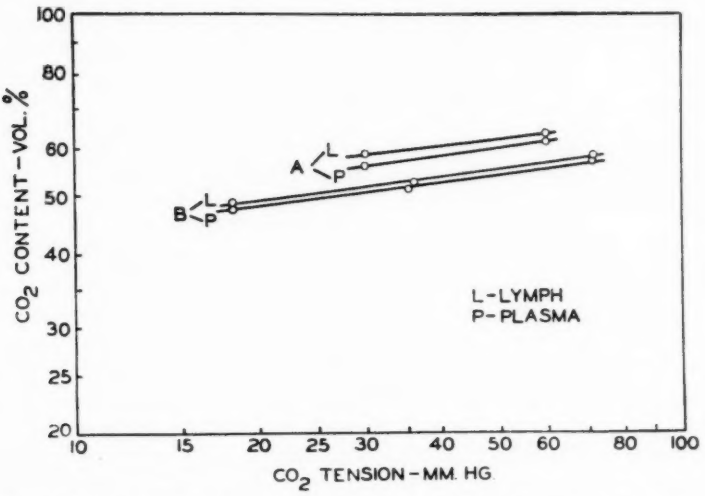

Fig. 1. Carbon dioxide dissociation curves of lymph and plasma. *A*, average of 15 experiments; *B*, typical experiment.

the limits of the experimental error. As we shall point out later, the saturation curve for lymph approximates a straight line when plotted on logarithmic coördinate paper. This means that both the lymph and plasma pH can be determined graphically on the same logarithmic pH chart. Lymph and plasma proteins were determined refractometrically.

DISCUSSION OF RESULTS. Figure 1 (B) shows typical dissociation curves for cervical lymph and separated plasma plotted on logarithmic coördinate paper. The values are based on the water contents of the fluids. It will be seen that the curves are approximately linear in shape, lie very close together, and have almost identical slopes. Table 1 gives values for the combining power of plasma and lymph of 15 animals at two points on the

dissociation curve. These figures were obtained by plotting the curves as in figure 1 (B) and determining the carbon dioxide contents at 30 and 60 mm. from the chart. These results are shown in figure 1 (A). The average difference in contents at these two tensions ($\Delta CO_{2_{60-30}}$) is 5.4 volumes per cent for plasma and 4.5 volumes per cent for lymph. The value of

TABLE 2

The carbon dioxide content and tension and pH of cervical lymph and separated plasma

DOG NUMBER	CO_2 CONTENT		CO_2 TENSION		pH	
	Plasma	Lymph	Plasma	Lymph	Plasma	Lymph
	volumes per cent		*mm.Hg*			
6	57.0	57.3	41.0	40.0	7.39	7.41
7		60.7		43.0		7.40
8	49.1	51.4	38.0	33.0	7.36	7.44
9	52.8	56.8	40.3	38.0	7.37	7.42
10	63.3	67.8	56.0	45.0	7.30	7.43
11	50.0	53.7	43.6	41.0	7.31	7.37
12	62.6	63.3	54.0	41.4	7.31	7.44
13	62.8	65.2	51.9	38.6	7.32	7.48
14		56.3		38.0		7.42
17		56.0		45.4		7.34
Average..	56.8	58.8	46.4	40.3	7.34	7.41

TABLE 3

The carbon dioxide combining power of plasma and cervical lymph during carbon dioxide administration

DOG NUMBER	CO_2 TENSION ALVEOLAR AIR	PLASMA		LYMPH	
		CO_2 content	CO_2 uptake per mm.Hg	CO_2 content	CO_2 uptake per mm.Hg
	mm.Hg	*volumes per cent*		*volumes per cent*	
15	38.8	57.5		59.9	
	85.9	67.0	0.202	67.9	0.170
16	36.8	62.3		65.3	
	91.1	73.5	0.206	73.1	0.144
18	41.2	58.4		60.0	
	90.4	68.5	0.205	69.6	0.195

$\Delta CO_{2_{60-30}} = 5.4$ of plasma is in good agreement with that of 5.6 which Eisenman found for human serum and is in exact agreement with the value of 5.4 which she found for cat's blood. We see from these charts that at a given tension of carbon dioxide, the lymph has a slightly higher combining power than plasma; while for a given change in tension, the lymph takes up slightly less carbon dioxide than the plasma.

Table 2 gives average values for the carbon dioxide content and tension and pH of separated plasma and cervical lymph of 10 animals. The carbon dioxide content of lymph varies from 51.4 to 67.8 volumes per cent with an average value of 58.8 volumes per cent. The carbon dioxide tension in the lymph ranges from 33.0 to 45.0 mm. of mercury and the average value is 40.3 mm. The average lymph pH was found to be 7.41 with a variation of 7.34 to 7.48. When compared with the corresponding pH value of 7.34 for separated plasma, it will be seen that the lymph is slightly more alkaline than the plasma.

Table 3 shows the results of three experiments in which the animal was permitted to breathe a gas mixture containing approximately 10 per cent carbon dioxide. It will be seen that the carbon dioxide uptake per millimeter of carbon dioxide tension is in every case slightly higher for plasma than for lymph.

SUMMARY

1. The carbon dioxide content and combining power and pH of cervical lymph of dogs have been determined.

2. The carbon dioxide dissociation curve of lymph was found to be a straight line when plotted on logarithmic coördinate paper.

3. For a given tension of carbon dioxide, the combining power of lymph is slightly greater than that of plasma.

4. The average difference between the carbon dioxide contents of lymph saturated at 30 and 60 mm. of carbon dioxide tension is 4.5 volumes per cent as compared with a corresponding value of 5.4 for separated plasma.

5. The average carbon dioxide content of the lymph of 10 dogs was found to be 58.8 volumes per cent.

6. The average pH of cervical lymph was found to be 7.41 with a corresponding value of 7.34 for separated plasma.

The authors are indebted to Dr. Cecil K. Drinker for advice and assistance throughout the course of this investigation.

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EFFECT OF POSTURE AND PROLONGED REST ON THE CARDIAC OUTPUT AND RELATED FUNCTIONS

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The cardiac output has been measured in these experiments by an application of the foreign-gas principle for the determination of the arteriovenous oxygen difference from which the cardiac output may be calculated, according to the equation:

$$\left.\begin{array}{c}\text{Cardiac output (liters of}\\ \text{blood per minute)}\end{array}\right\} = \frac{\text{Rate of } O_2 \text{ consumption (cc. per min.)}}{\text{A-V difference (cc. of } O_2 \text{ per liter of blood)}}$$

The principles of the procedure and the method of calculation have been covered in detail by the writings of Krogh and Lindhard (1), (2) and Marshall and Grollman (3), (4).

The method is based on a comparison between the amount of oxygen and the amount of foreign gas absorbed by the mixed venous blood during its passage through the lungs. The validity of the results depends among other things upon the completion of the procedure before any appreciable amount of blood which has once been exposed to the foreign gas can complete a circuit and return to the lungs for a second time. The other technical difficulty is to obtain a homogeneous mixture in the gaseous system with which the blood leaving the lungs is assumed to be in equilibrium. This homogeneity of composition must be attained before the first sample is drawn, and with sufficient speed to allow time for comparing the amounts of oxygen and foreign gas absorbed before any appreciable recirculation occurs. The error due to recirculation has been estimated by Grollman as being quite small and negligible partly because of the compensating error due to quickening of the circulation by the deep breathing and the resultant slight increase of the oxygen content of the mixed venous blood. In 1932 Hamilton, Spradlin, and Saam (5) reported an enquiry into the basis of the acetylene method of determining the cardiac output, and by means of animal experiments, emphasized the error due to the

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venous return of acetylene. The present writer after using the Marshall-Grollman procedure for one year (6) has felt it advisable to obtain more accurate information on this question in human subjects.

Two normal young men were used as subjects. The experiments were performed in the forenoon, one to four hours after breakfast. The subject sat in a chair for 10 minutes after which the rate of oxygen consumption was measured by means of a Sanborn-Benedict metabolism apparatus. The subject then rebreathed from a rubber bag containing an acetylene mixture, expiring to residual air before breathing from the bag, emptying the bag on each inspiration and breathing out as far as possible on each expiration. During the rebreathing several alveolar samples were drawn into evacuated tubes each at the end of a given expiration. The breathing and sampling were directed by an accurately timed phonograph record. In several experiments the inspired mixture was divided into two portions, the contents of the side bag being inspired first, the contents of the front bag following. This was done in order more quickly to attain a homogeneous mixture in the lung bag system (see below).

If and when any appreciable amount of acetylene-containing blood returns to the lungs for a second time, such recirculation will result in a depression in the rate of diffusion of acetylene from the lungs into the blood, and a concomitant rise in the calculated A-V difference. If the blood flow through the lungs during the rebreathing is fairly constant, the rate of diffusion of acetylene from the lungs into the blood should always be proportional to the concentration of the gas in the lungs. Neglecting slight changes in total volume of the gaseous system, the rate of decrease of the acetylene concentration should follow the equation,

$$-\frac{dC}{dt} = KC$$

where C is the concentration of the acetylene in the lung bag system at the time t, and K is the diffusion constant. For any interval of time t (between t_1 and t_2) one obtains

$$\frac{\log C_1 - \log C_2}{t} = 0.4343K$$

Accordingly, if the rate of diffusion of acetylene is proportional to the concentration, the latter should follow an exponential or parabolic curve against time as the abscissa, and the logarithms of the acetylene concentrations should follow a rectilinear curve.

The results obtained in ten experiments on two subjects demonstrate a rise in the calculated A-V difference as the breathing continues, and a fall in the rate of diffusion of C_2H_2 from the lungs into the blood. A typical

experiment is summarized in table 1. The logarithms of the C_2H_2 concentrations charted against time as the abscissa, in all experiments indicate a decrease in the slope of the curve occurring within ten to twelve seconds after the rebreathing has begun. Since the slope of the curve indicates the rate of diffusion of the gas, the change in slope of the curve indicates the time at which recirculation begins in appreciable amounts. Since the normal complete circulation time is about 22 seconds (7) the present findings indicate that the deep rapid breathing has approximately doubled the rate of blood flow through the lungs.[2] This conclusion is confirmed by calculation of the volume of oxygen or of acetylene removed from the lung-bag system by the blood during the first ten seconds, before recirculation

TABLE 1

Results of typical rebreathing experiment

	BREATH NUMBER					
	2	4	6	8	10	12
CO_2	4.69	5.89	6.54	6.86	7.17	7.44
C_2H_2	12.53	10.87	9.58	8.73	7.90	7.20
O_2	19.77	18.85	17.91	17.21	16.32	15.33
N_2	63.01	64.39	65.97	67.20	68.61	70.03
Time interval (second)		5–10	10–15	15–20	20–25	25–30
A-V difference		56.5	63.4	63.8	71.1	80.6
K (diffusion constant)		0.02842	0.02526	0.01859	0.01998	0.01856
Rate of C_2H_2 diffusion		125.1	111.2	80.13	84.38	76.56

Bag contents before rebreathing:

	cc.
O_2	270
C_2H_2	600
Air	1530
Total	2400

has begun. To account for the volumes of each gas removed, the mixed venous blood must pass through the lungs at a rate of 10 to 12 liters per minute. The promptness (10–12 sec.) with which recirculation begins necessitates a very rapid attainment of uniformity in the gaseous system to permit the drawing of the first sample within 3 or 4 seconds, leaving a period of five or six seconds during which the gaseous diffusion from the lungs into the blood may be measured.

Two methods of achieving this result have occurred to the writer. One is to wash out the dead space into a side tube after the first deep inspira-

[2] Gladstone, S. A. and Dack, S. Proc. Soc. Exp. Biol. Med. **32**: 1315, 1935. Gladstone, S. A., ibid., p. 1319.

tion, using about 800 to 1000 cc. to accomplish this (8), then to turn the valve so that the remainder of the first deep expiration enters the breathing bag and consists entirely of alveolar air. The other method to *approximate* uniformity is to start the rebreathing after having expired to residual air, by inhaling in succession the contents of two separate bags, the composition of which has been adjusted so that the mixture in the second bag (the last portion of which fills the dead space) approximates in gaseous composition the expected alveolar air resulting from a mixture of the contents of the first bag and the residual lung air. In the technique to be described both these methods have been incorporated, the advantage being that the error due to possible incomplete washing of the dead space will be reduced to a minimum because the gaseous composition of the dead space has been previously made to approximate that of the alveolar air.

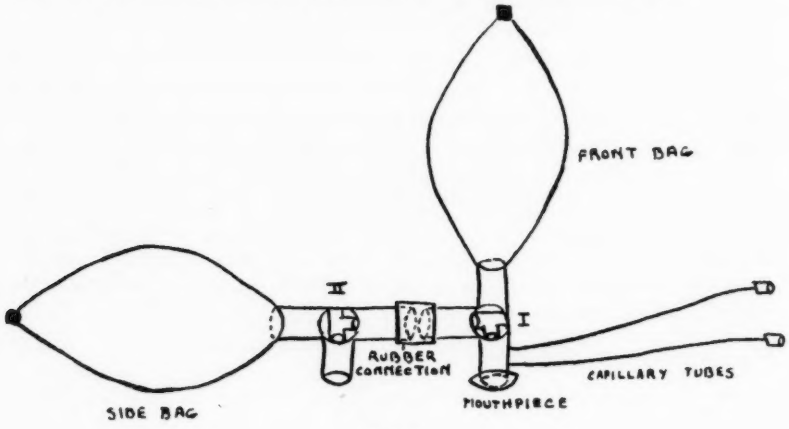

Fig. 1

METHOD. The apparatus consists of two three-way aluminum valves (bore 2 cm.), two rubber breathing bags (capacity 4 liters) connected as shown in the diagram (fig. 1) and supported on iron stands so that the subject with his lips over the mouth piece will have one bag directly in front, and the other bag to his left. Brass or lead tubes 15 cm. in length and of capillary (0.5 mm.) bore permit the drawing of samples from the mouth side of the valve into evacuated sampling tubes. After the valves have been turned to close both bags, the gases are introduced as follows:

Front bag cc.		*Side bag* cc.
825	Air	1300
55	CO_2	50
120	C_2H_2	500
	O_2	150
Total....1000		Total....2000

A vital-capacity spirometer may be used for measuring the volume of air introduced, and a 250 cc. glass graduated syringe for measuring the other gases. If commercial acetylene is used, it must be washed to remove the traces of acetone and other impurities as recommended by Grollman (4). In preparing the mixtures it is preferable first to introduce the required amount of air into each bag to avoid at any time a pure oxygen-acetylene mixture. In normal subjects with a vital capacity of 3500–4000 cc. the volumes indicated are satisfactory. If the vital capacity rises much above 4000 cc., the volume of gas in the front bag may be increased, the percentages being kept the same.

The rebreathing. The subject with his lips over the mouthpiece breathes quietly through his nose until the spring nose-clip is applied and the subject exhales through the mouth to residual air, the air leaving through the side arm of valve II (fig. 1). After the subject has expired to residual air, valve II is turned so that the subject inspires the contents of the side bag. Immediately the side bag is empty, valve I is turned so that the contents of the front bag may be inhaled. When the front bag has been emptied, valve I is again turned to permit approximately the first liter of the ensuing expiration to be discarded into the side bag, the remainder of the expired air being then directed by another turn of valve I into the front bag. During this procedure the subject has taken a deep inspiration, emptying both bags, and a deep expiration, the first portion of which has been discarded. The operator has turned valve II once, and valve I three times. This preliminary period can be finished in 3.5 to 4.0 seconds after a little practice on the part of the operator and without undue haste on the part of the subject, whose only task after expiring to residual air is to inhale deeply and then exhale deeply. At the end of the latter expiration the first alveolar sample is drawn. The subject then takes two complete deep breaths out of and into the bag, emptying the bag on each of the two inspirations, and then breathing out as far as possible. These two complete respiratory cycles should take about 5 seconds. At the end of the second deep expiration, the second alveolar sample is drawn. The total duration of the test, i.e., the interval between the first inspiration of the foreign-gas mixture and the drawing of the second gas sample is $8\frac{1}{2}$ to 9 seconds. Errors due to recirculation of blood containing the foreign gas will become appreciable at ten seconds or shortly thereafter.

Mixing. Before applying the method described it is well to test the adequacy of the technique for attaining uniformity of gaseous composition prior to drawing the first sample. If the gaseous system is of heterogeneous composition immediately before the drawing of the first sample, such heterogeneity may be demonstrated by simultaneously drawing the first sample from both the proximal and distal ends of the rebreathing bag, and calculating the A-V difference from either of these as the first sample

paired with the usual second sample drawn after the last breath. If homogeneity of gaseous composition has not been attained the A-V differences thus calculated should show wide divergence. If homogeneity has been attained, the two samples drawn from opposite ends of the bag should show only a slight difference because the mouth sample has left the lungs a fraction of a second later than the distal sample, and since gaseous diffusion has continued during that time, one will find a slightly higher CO_2 and lower O_2 and C_2H_2 concentration in the mouth sample. These changes, resulting from diffusion across the alveolar epithelium should not affect the calculated A-V difference.

Experimental. Seven determinations of the A-V difference were made on three subjects, reclining in a deck chair, one to two hours after break-

TABLE 2

Effect of drawing first alveolar sample from opposite ends of rebreathing bag

SUBJECT	PULSE RATE PER MINUTE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERNCE OF BLOOD		DIFFERENCE BETWEEN A AND B	CARDIAC OUTPUT	
				A	B		I	II
		mm. Hg	*cc. per minute*	*cc. per liter*	*cc. per liter*		*liters per minute*	*liters per minute*
A. L.	64	108/65	256	49.7	53.9	4.2	5.2	4.8
A. L.	76	106/75	284	50.4	49.7	0.7	5.6	5.7
A. L.	68	112/72	258	51.3	52.3	1.0	5.0	4.9
S. D.	76	115/70	259	43.8	50.2	6.4	5.9	5.2
S. D.	70	105/72	247	43.6	46.0	2.4	5.7	5.4
S. A. G.	88	116/80	252	43.1	45.8	2.7	5.9	5.5
S. A. G.	88	120/76	270	50.3	50.1	0.2	5.4	5.4
Average						2.5	5.5	5.3

fast. The first sample was simultaneously drawn from opposite ends of the rebreathing bag. In column A (table 2) are given the results of the conventional samples. In column B, the additional first distal sample has been paired with the second sample. The average difference of about 5 per cent in the two sets of determinations may be attributed to the combined errors of inadequate mixing and gas analysis, and since the latter alone may easily account for the differences observed, one may assume that the error due to inadequate mixing in the method described is either negligible or non-existent.

RESULTS. The method as described has been applied in 34 consecutive determinations to study the effect of posture and prolonged rest on the cardiac output and related functions in six normal young men, fasting and at rest. Each subject was studied while sitting upright in a straight-back

chair, and usually on the following day while reclining in a deck chair. Since the findings are similar in all subjects, a detailed protocol is presented for the first subject (table 3) and the findings for the group are summarized in tables 4 and 5. The results indicate a difference in all circulatory functions listed (not the rate of O_2 consumption) when the two positions studied are compared. In the sitting position the pulse rate, systolic pressure, diastolic pressure, and A-V difference are distinctly higher, while the

TABLE 3

Subject, A. L. Male, aged 33 years. Height, 166 cm.; wt. 57 kgm.

TIME	PULSE RATE PER MINUTE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT	REMARKS
a.m.		*mm. Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*	
8:50						2/6/35 (seated in
9:25	62	112/85				chair)
9:45	63	110/82				
9:56			215			
10:00	61	110/85				
10:07				65.4	3.3	
10:37			222			
10:40	61	110/85				
10:45				68.3	3.3	
11:20			222			
11:30	57	110/85				
11:31				65.4	3.4	
9:00						2/7/35 (reclining
9:50	58	100/68				in deck chair)
10:05			194			
10:10	60	102/65				
10:15				41.2	4.7	
10:47			206			
10:55	57	105/80				
11:00				58.0	3.6	
11:34			217			
11:40	55	105/75				
11:55				58.7	3.7	

cardiac output, systolic output, and pulse pressure are distinctly lower when compared with the results found in the recumbent position. As rest is prolonged in either position, the pulse rate and cardiac output progressively decrease. It is significant that the changes in systolic output whether produced by postural difference or by prolonged rest are accompanied by similar changes in the pulse pressure (9).

Discussion. The present method for determining the arterio-venous

oxygen difference and cardiac output does not differ in principle from the nitrous oxide method of Krogh and Lindhard (2), but follows more closely the rebreathing procedure of Marshall and Grollman. The essential feature of the present method is its termination within 8.5 to 9 seconds to avoid the error due to recirculation which begins within 10 seconds or promptly thereafter, as demonstrated by the rebreathing experiments here reported. To complete the procedure within 10 seconds a rapid

TABLE 4

Effect of posture and prolonged rest on cardiac output (liters per minute)

SUBJECT	SITTING			RECUMBENT		
	I hr.	II hr.	III hr.	I hr.	II hr.	III hr.
A. L.	3.3	3.3	3.4	4.7	3.6	3.7
S. D.	4.9	3.4	3.2	4.2	4.1	4.6
S. A. G.	4.3	3.4		5.3	4.4	
A. P.	4.7	4.7	3.9	4.1	4.8	3.8
S. Z. S.	3.3	3.7	2.9	4.4	4.0	4.5
J. B.	3.5	3.3	3.1	3.8	4.1	3.7
Average	4.0	3.6	3.3	4.4	4.2	4.1

TABLE 5

Averages of circulatory functions in six subjects as affected by posture and prolonged rest

FUNCTION	SITTING			RECUMBENT		
	I hr.	II hr.	III hr.	I hr.	II hr.	III hr.
Pulse rate per minute	66	65	63	60	59	58
Systolic blood pressure	114	113	113	104	107	107
Diastolic blood pressure	88	88	90	71	75	75
Oxygen consumption, cc. per minute	217	214	216	210	219	218
A-V difference	56	60	66	49	53.6	54.4
Cardiac output, liters per minute	4.0	3.6	3.3	4.4	4.2	4.1
Systolic output, cc	61.3	59.5	51.0	71.8	70.3	70.6
Pulse pressure	26	25	23	33	32	32

method for attaining homogeneity of gaseous composition within 3.5 to 4.0 seconds has been imperative. Such a method has been described and its adequacy tested. The writer believes that after removal of the error due to recirculation, the foreign-gas method is rendered more accurate as to absolute values obtained, and more sensitive in picking up small changes in arterio-venous oxygen difference. This belief is strengthened by a comparison of the results here reported with those obtained by Grollman

(10) who found no change in the cardiac output in different postures, although practically all other workers using different methods have reported results which agree with the present observations (11).

The finding of a higher cardiac output in the recumbent position, and a decreasing cardiac output as rest is prolonged in either the sitting or recumbent position cannot be attributed to any metabolic differences because no consistent differences in metabolic rate were observed. The observed changes probably result from the better venous return to the heart in the recumbent position, and the decreased venous return as muscular relaxation is increased by prolonged rest.

The effect of posture on the cardiac output (and related values) and especially the effect of prolonged rest indicate the importance of accurately defining these factors in the attempt to establish normal values or in the comparative study of abnormal conditions produced by experiment or by disease.

SUMMARY

A method based on the foreign gas principle for determination of the arteriovenous oxygen difference (and cardiac output) has been described. Its distinguishing feature is its termination within ten seconds in order to avoid the error due to recirculation of appreciable amounts of blood, which has been demonstrated to occur at that time or promptly thereafter.

The method as applied in 34 consecutive determinations in six normal young men has demonstrated a cardiac output 10 to 25 per cent higher in the recumbent as compared to the sitting posture. In either position, although basal conditions may be attained in one hour as indicated by the constancy of the rate of oxygen consumption, the cardiac output progressively decreases as rest is prolonged for a second and third hour. These changes run parallel with the observed changes in pulse rate and pulse pressure, the latter following closely the systolic output.

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THE EFFECT OF LACTOGENIC HORMONE PREPARATIONS ON THE BLOOD SUGAR LEVEL OF RABBITS AND MONKEYS

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The observations of Houssay and Biasotti (1931), Barnes and Reagan (1933), Baumann and Marine (1932) and others that some anterior pituitary factor is involved in carbohydrate metabolism have added an additional rôle to the array of functions attributed to the anterior lobe. The possibility that the blood sugar-raising factor of the anterior lobe might be identified with some recognized principle has been considered, but very little evidence has appeared concerning such an alliance. The extracts used by the above workers were rather crude preparations and undoubtedly represented a complex of pituitary factors. The partially purified growth hormone extracts used by Evans, Meyer, Simpson, and Reichert (1933) would appear to fall in the same category. Lucke, Heydemann, and Duensing (1933) report that their thyrotropic extract does not induce glucosuria. Ferrill, Rogoff, Barnes and Scott (1934), as well as others, have presented evidence which suggests that the hypophyseal relation to carbohydrate metabolism may be exerted by way of the adrenals. However, insofar as we are aware, no reports have appeared concerning the effects of a purified adrenotropic extract upon carbohydrate metabolism.

We have been attracted by the possibility that the blood sugar-raising factor might be identical with the lactogenic hormone since the secretion of milk demands the utilization of quantities of sugar. Support was accorded this possibility by the observation that certain female dogs injected with a crude pituitary extract which induced glycosuria also lactated.

We have sought to investigate this question on monkeys and rabbits by means of various anterior pituitary extracts of proven lactogenic potency. The Schaeffer-Somogyi (1933) method of sugar determination was used in the estimation of blood and urine sugars. The monkey urines were twenty-four hour samples while the rabbit urines were obtained by catheterization.

[1] From the Dairy Husbandry Department, Agricultural Experiment Station, University of Missouri, Journal Series Paper no. 404.

The monkeys used were all sub-adult female macaques. Two were otherwise untreated and two had been totally depancreatized. The diabetes of the latter two animals was controlled by the daily injection of oestrin (Nelson and Overholser, 1934). Two extracts of known lactogenic potency were tested on these animals. Extract B contained one rabbit unit[2] of lactogenic hormone in 3 cc., while extract A[3] had a potency of one rabbit unit per 15 mgm. As made up in solution the potency of the latter was one unit in $1\frac{1}{2}$ cc. Although we have not studied these extracts for their content of all of the supposed pituitary hormones we have found no significant gonadotropic (follicle-stimulating) or thyrotropic effects. Riddle and his co-workers (1933), who have examined the effects of prolactin extracts prepared by a similar method, state that there is little or no contamination of such preparations by the other recognized pituitary principles. The extracts used in the present study when injected in the monkeys in amounts of 3 to 6 cc. daily over periods of 4 to 7 days induced no significant changes in the blood sugar level and no evidence of glycosuria. Lactation did not occur in these animals despite the potency of the extracts. Since the mammary glands of these monkeys were not developed sufficiently to support lactation this finding was not surprising. Each of these animals later showed hyperglycemia and glycosuria following the administration of a crude alkaline extract of sheep pituitaries or an acetic acid extract of beef hypophyses (Nelson and Overholser, 1934). The sheep pituitary extract contained appreciable amounts of lactogenic hormone, but the beef gland extract showed no activity when tested on the pseudopregnant rabbit.

In studying the effects of extracts A and B upon the rabbit we have used pseudopregnant and oestrous animals. The animals were made pseudopregnant by the intravenous injection of Antuitrin-S.[3] This treatment, as is well known, induces ovulation and corpus luteum formation. The mammary glands of such animals undergo marked proliferation and are completely responsive to the lactogenic hormone. The oestrous animals represent isolated adult females receiving no preparatory treatment. The value of using pseudopregnant animals in the present study is apparent since we thus were able to check the lactogenic potency of the extracts while we were determining their capacity to elevate the level of the blood sugar. The oestrous animals, while not as suitable for the study of the

[2] The rabbit unit is defined by Gardner and Turner (1933) as the minimum amount of extract which injected during a period of seven days at daily intervals is required to induce a plus three or plus four lactation in pseudopregnant rabbits. We have used five rabbits on a given level of hormone and demanded the above response in at least three of the five animals.

[3] This material was supplied to us through the kindness of Dr. Oliver Kamm of Parke, Davis & Co.

lactogenic hormone, might be expected to respond to any blood sugar-elevating activity present in the extracts.

The usual procedure employed on the rabbits was the injection of a given amount of hormone over a period of 6 to 10 days. The mammary glands were examined at the end of the experiment and the lactation rated according to the method of Gardner and Turner (1933). At least one and often two blood sugar determinations were made on each animal prior to the initiation of treatment. Thereafter, several determinations were made on each animal during the test period.

Seven pseudopregnant and three oestrous animals were tested on extract A. The details of treatment and blood sugar findings for these animals are shown in table 1. It will be noted that with the exception of

TABLE 1

Data on rabbits injected with extract A

RABBIT NUMBER	DAILY DOSE	BLOOD SUGAR VALUES (MGM. PER CENT)								
		Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Days 7–10	Lactation rating
Pseudopregnant										
1	½ Rb. U.	88; 75	95	90	85	73		95		Plus 4
2	1 Rb. U.	105; 90	90		115	75	88		110; 95	Plus 4
3	1 Rb. U.	82	85	95	80	72		88		Plus 4
4	2 Rb. U.	75; 70	98	115	110	78	85	105		Plus 4
5	2 Rb. U.	72	80	65	75		70	85	78; 80	Plus 3
6	3 Rb. U.	80; 92	72	72	85	80	88	75		Plus 4
7	3 Rb. U.	90	78	85	75	70		88	105; 88	Plus 4
Oestrous										
8	1 Rb. U.	80	95	78	88	110	85	88		Slight
9	2 Rb. U.	75	82	68	72	95	85	88	92; 82	Plus 1
10	3 Rb. U.	95; 82	80	88	95	100	72	80		None

rabbit 4 there is no evidence that the extract had any tendency to elevate significantly the level of the blood sugar. Although rabbit 4 showed a rather consistently increased blood sugar we are inclined to consider this single instance as of doubtful significance. As a whole the individual readings are of the order that one might expect to find in a similar group of untreated rabbits. In several of these rabbits a number of urine samples were obtained by catheterization. None of these urines showed evidence of sugar.

As was to be expected the pseudopregnant rabbits all showed excellent lactation. The oestrous animals showed a variable degree of lactation. Rabbit 9 had a fair plus one (ducts filled with milk) lactation, while rabbit 8 showed only a scanty secretion and rabbit 10 did not lactate. Further-

more, the lactogenic hormone did not induce any detectable increase in mammary gland proliferation.

Extract B was tested on six pseudopregnant and three oestrous animals with results similar to those found for extract A. In no instance did the blood sugar readings vary significantly from the normal range. The degree of lactation observed in these animals was of the same order as has been noted for the preceding group.

SUMMARY

In the present study preparations of the lactogenic hormone which were believed to be relatively free of other recognized pituitary principles failed to induce significant alterations in the blood sugar level of monkeys and rabbits.

Four monkeys showed no elevation of the blood sugar level and no glycosuria during the test periods. Thirteen pseudopregnant and six oestrous rabbits were treated for periods up to 10 days. No significant alteration in the blood sugar level occurred and no sugar was found in the urines examined.

It would appear from these studies that the lactogenic hormone is not allied with the blood sugar-elevating factor of the anterior pituitary. Previous studies have shown that lactogenic hormone preparations may be relatively free of the growth, thyrotropic, adrenotropic, and follicle-stimulating factors.

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